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THE CHILD WITH POTENTIAL HEART DISEASE*

C G KERLEY, MD
NEW YORK

Organic acquired heart disease in the child is one of childhood's diseases in which practically no progress has been made in alleviation or cure in the past forty years which covers the time that I have been actively engaged in the practice of pediatrics, and this in spite of the establishment of associations for the study of heart disease in children, in cardiac clinics and cardiac homes. I do not discredit in any sense the effort to improve the condition of the child who has heart disease, but when our best has been accomplished, the patient still has a defective organ with all that it implies. Our prophylactic attempts have rested largely in the eradication of apparently infected foci, but this is no insurance against heart disease, as all who treat children on any considerable scale are aware. If every child from the third to the tenth year of age could pass through this period free from infected foci, there would be fewer cases of acute endocarditis but the disease would be by no means eradicated. Every year patients with endocarditis come to my attention who have received adequate focal treatment before the development of the endocarditis, this fact the literature on the subject emphasizes. In order to make an impression on the prevalence of heart disease in children we must appreciate that this is a problem intimately connected with the early years of life, it is well known that certain family groups possess a diminished resistance to the invasion of the tubercle bacillus. Family susceptibility we are aware exists for other diseases.

In certain children there exists a physical state, a physiologic entity, which supplies an inherently favorable soil (which may not be experimentally produced) for the development of infecting agencies that may cause rheumatic endocarditis, and this type of endocarditis is responsible for more than 90 per cent of the cases of organic heart disease in children. The child with the inherently favorable soil is a child with potential heart disease, in that he is more vulnerable than others to the infecting micro-organisms. If all children with diseased teeth, infected sinuses and infected tonsils developed endocarditis, few would escape. As it is, few of the great family of children develop the disease and these are the unfortunates whose bodies supply the favorable soil.

A vast number of patients with heart disease have come under my observation in private, hospital and outpatient practice, it is rare that the early history was

not sufficiently suggestive to stamp the case as potentially cardiac. Months and perhaps years before heart disease developed the children with potential heart disease show warning signals, and what are the significant signs? Muscle pain and stiffness, sometimes joint pains, pain in the extremities by day and often complained of by night. The complaint repeatedly of tired legs is significant in a well child. Children who sleep restlessly, who toss about much in their sleep, have been made to rest normally by the use of salicylates. All children who complain repeatedly of muscle and joint pains, particularly on wet and so-called damp days, stand out prominently in my mind as potentially having heart disease. In such cases an immediate removal of the tonsils, regardless of their appearance, is demanded. The teeth should be passed on by a roentgenologist, and there should be competent sinus attention. No one may say in looking at a tonsil that it is not diseased. The small, innocent appearing buried tonsil may be badly diseased and most dangerous.

In addition to the eradication of possible foci of infection, I put these predisposed patients under the administration of salicylic acid in some form: sodium salicylate usually, and methyl salicylate (oil of wintergreen) occasionally. The drug is given in sufficient amount to control the pain and discomfort and later it is given interruptedly—five or ten days or more out of the month for months and perhaps years, and its use is resumed in a vigorous fashion at the first indication of a return of the muscle pain or soreness.

A child with rheumatic fever of the acute articular type is preeminently one with potential heart disease. For prophylactic cardiac purposes, such patients must be given large doses of the salicylates while the disease is active and interruptedly thereafter for an indeterminate period after the same fashion every patient with chorea is looked on and treated as potentially having heart disease. Parents are acquainted with the danger confronting such children and are told that the child should be under medical supervision until he is 12 or more years of age.

It has been asserted that the use of salicylic acid is unscientific, that there is no proof of bactericidal effects, which I admit. So much attention has been paid to antiseptic agents in infection that we forget that our beneficial results in the use of a remedy may be due to its ability to establish a protective tissue resistance to the infection in question. The child I have described has a diminished resistance to the type of infection the outcome of which may be endocarditis.

As a medical student in Vienna, thirty-six years ago, I recall that Kassowitz was a most ardent advocate of the use of cod liver oil in rachitis. Norse fishermen had given it to their children a few hundred years before Kassowitz, and how long is it that we have known even a little as to how it acts?

* Read before the Section on Diseases of Children at the Seventy Ninth Annual Session of the American Medical Association, Minneapolis, June 15, 1928.

Decoctions of cinchona bark were used for the treatment of malaria hundreds of years before the plasmodium was discovered, and the use of mercury antidescribes the discovery of the spirochete many hundreds of years. I admit that the use of salicylic acid as a prophylactic agent against endocarditis is empiricism and impossible of scientific demonstration. "Medicine is in part a science, it is also an art that uses science as far as it can, but uses useful facts regardless of their origin."¹

A discussion of heart disease is not a part of this contribution, but the question arises, Does the heart in the performance of its function in any way indicate that its integrity is threatened? Physicians and parents are often much disturbed by the irregular heart action of the small child and more disturbed by the rapid heart of the highly emotional overworked school girl in whom there may be pulse irregularities, the heart pounds, and there may be a visible cardiac impulse. The physician thinks that the heart is involved, auscultation adds to the confusion in that it may reveal what Cabot defines as a presystolic gallop arrhythmia in which an extra sound occurs just before the usual first sound which is never clearly defined, with accent on the second half and which passes as a murmur. The presence of a pathologic murmur is further established in the minds of the examiner by the presence of an apparently accentuated second sound. It is not appreciated that the pulmonic second sound in children is almost always louder than the aortic. Such a rhythm may occur in any heart that is excited or overworked. I find it a very frequent occurrence in patients of the type under consideration. Such patients are often diagnosed as having heart disease or potential heart disease but they do not have heart disease in any pathologic sense. The phenomenon disappears shortly under rest and the removal of emotional surroundings. The cardiac phenomenon is just one of the manifestations of nerve imbalance due to indifferent causes, which I may not take up at this time.

CONCLUSION

There is a type of child that is particularly susceptible to the infecting agencies that cause heart disease. Such a child possesses what is termed a favorable soil and may be recognized and belongs to one of three groups.

Group 1 Those who have or who have had rheumatic fever.

Group 2 Those who have or who have had chorea.

Group 3 Those who complain repeatedly of pain in the extremities which may not be accounted for by trauma or disease. A particularly susceptible child may be classified as belonging to the three groups. Heart disease in children so afflicted may be prevented in many by the eradication of diseased foci, first with a persistent and later with an interrupted use of salicylic acid alone or combined with an alkali.

When physicians appreciate the dormant possibilities behind the 'growing pains,' heart disease will be appreciably lessened.

132 West Eighth-First Street

ABSTRACT OF DISCUSSION

DR. WILLIAM A. MULBERN, Augusta, Ga. All well informed pediatricians I feel are fully cognizant of the cardiac dangers that lurk in myalgia (growing pains), mild arthritic pains and chorea, especially if these clinical manifestations occur in a child from 5 years of age to puberty. It is the general

practitioner who does not fully realize that the common manifestations of acute rheumatic infection are myalgia, mild arthritic pains, chorea and endocarditis or pancarditis. Rightly, Dr. Kerley calls attention to the potential cardiac child, for when muscular pains exist in children they are usually indications of rheumatic infection and endocarditis, another manifestation of this infection, is to be expected. The profession has at its command preventive measures, as regards heart involvement, but the results appear not to have been brilliant. However, I feel convinced that the profession has been and is accomplishing something worth while along this line. The preventive measures to which I refer are (1) a clearer conception of rheumatic infection and its frequent cardiac manifestation, (2) a keener desire to remove infected foci, thereby protecting the heart, (3) the establishment of cardiac clinics and homes, which are educating the mothers to the dangers of heart involvement in mild rheumatic infection, (4) better nutrition of babies and children, thereby raising their vital forces that they may better resist all infections, (5) physical rest in comfortably warm rooms when mild rheumatic pains manifest themselves, and confinement to bed when fever accompanies them, and (6) the more intelligent and freer use of salicylates. All these measures, I feel quite positive, are preventing cardiac involvement in some acute rheumatic cases. In regard to heredity, I am thoroughly in accord with Dr. Kerley. I think that there does exist a physical state, a physiologic entity, that supplies the individual with that inherent lowered resistance to the organism, whatever it might be, that causes acute rheumatic infection. As regards the removal of tonsils in all cases in which rheumatic infection exists, I am inclined to believe that the question is debatable. When the tonsils can be incriminated they should be removed but in all instances I do not feel quite sure that much is accomplished by tonsillectomy.

MODERN DIAGNOSIS

THE SECOND ALPHA OMEGA ALPHA
ANNUAL LECTURE *

JAMES B. HERRICK, M.D.
CHICAGO

Successful diagnosis depends on a working familiarity with the facts and principles of medicine and with the rationale and technic of many laboratory and instrumental procedures. The mass of medical knowledge has become so huge, the procedures so numerous and often so complicated, the contacts with sciences cognate to medicine so frequent and intimate as to make diagnosis today, while far more accurate, much more difficult than it used to be.

Formerly even through the middle ages, diagnosis was largely a matter of acquaintance with tradition and the fathers of medicine, a speculative, philosophic contemplation carried on nearly as well by solitary musing in the study as at the bedside. Later, cases were carefully observed and clinical pictures drawn, such as that of chorea by Sydenham. The era of physical diagnosis, with the correlation of its observations with those of pathologic anatomy as expounded by Morgagni, lasted several decades from the time of Laënnec in the early nineteenth century, it is not yet entirely a memory. With the microscopic examination of tissues—including the blood—and the new science of bacteriology, the diagnostic laboratory of pathology and bacteriology evolved. Body fluids and gases were analyzed by chemical methods. Scores of instruments of investigation were devised. The x-rays in the nineties revolutionized diagnosis, threatening to overthrow physical examination and greatly altering the interpretation of symptoms. New disease complexes were

¹ Pub. W. A. The Importance of Being Historically Minded
J. A. M. A. 50:2079 (Dec. 17) 1927

* Read before the Alpha Omega Alpha, Minneapolis, June 14, 1928

described, disturbance of function as well as anatomic change was stressed, and many refinements as to physical observations and symptoms were noted. Altogether a type of diagnosis has come into being, wonderfully different from that of two or three centuries ago. This depends so much on an intimate acquaintance with the appalling number of facts concerning disease and with technical manipulations as to defy the efforts of any ordinary mind to become a master in all departments. Medical diagnosis has long ceased to be a one man job.

There is another feature that, not always recognized, makes it difficult for one man alone to make the diagnosis. To name a disease is not everything. The modern diagnostician is expected to understand what is implied by this name. He must view the subject from many angles, etiologic, anatomic, physiologic, clinical. As a matter of fact, no two physicians view a case exactly alike though they may agree as to diagnosis. Each sees a different picture with a foreground stress on different features. To illustrate. Several physicians may examine a patient and agree that his disease is typhoid. But to one the diagnosis typhoid brings up a picture of the germ of the disease, of bacteremia with toxemia. Another sees the anatomic changes, the ulcers, the enlarged glands, the spleen. Still another emphasizes symptoms, i. e., the fever, the slow pulse, the spots, or he may visualize a possible hemorrhage or perforation. Prognosis or treatment may be the thought of a fourth—specific therapy, the value of drugs, the proper diet. Or, again, the social and economic features may be considered, the danger to the others in the family or the community, or the monetary loss. While all such thoughts may not consciously go through the mind of the physician, he must recognize these and other factors if he is to be the well informed physician who makes a comprehensive, scientific diagnosis.

It is clear, then, that no one man can be a universal diagnostician. He must have help in any except the simpler, everyday cases. Where can the physician get this help? The answer has been already worked out—partially. First, clinical specialists have developed. For a long time such specialties as surgery, obstetrics, ophthalmology, neurology and dermatology have had recognition not alone by physicians but by lay people as well. The patient with a visible tumor of the breast or a disabling injury to a bone goes directly to the surgeon for diagnosis as for treatment. For a painful eye or a supposed neuralgia one goes to the ophthalmologist or the neurologist.

Secondly, laboratory and instrumental specialists, as microscopists, bacteriologists, chemists and roentgenologists, have appeared in great numbers. These men may have perfected themselves in the particular method of examination in the course of their clinical practice, e. g., the urologist, or they may, without active bedside practice, perhaps not even being graduates in medicine, class themselves as pure diagnostic specialists, e. g., chemists and bacteriologists, whose avowed vocation is to give expert opinion to physicians on some particular phase of disease. In the latter capacity they are often found as members of a group whose personnel may function as an independent diagnostic laboratory or as a part of the equipment of the up-to-date hospital.

Lastly there is the group *sensu strictiori*, often called the clinic—practitioners of various types with laboratory and instrumental experts, with free interchange of opinions and intensive departmental study of special features of disease. The group is still on trial.

Whether it will remain in some modified and improved form is a matter to be decided in the future.

One finds, therefore, that while the individual physician may be incompetent to use to the full the accumulated knowledge of disease, or himself to employ the many useful diagnostic aids furnished by the laboratory and by instruments, he may yet receive the needed help from consultation with colleagues who are specialists, from diagnostic specialists or from the so-called group, a combination of the clinical and laboratory workers.

Now there are dangers and difficulties in these methods. Though some of these have been pointed out by others, they will bear repetition.

1 The point of view of the clinical specialist is often narrow. He is apt to go at a case as do some unwise microscopists who omit the bird's-eye view afforded by the low power lens and use only the oil immersion. He thus misses the relations of parts and ignores things outside his own narrow field.

2 Technicians, often not physicians, are too prone to interpret observations in terms of disease, concerning the pathologic and clinical features of which they may know very little. And their interpretation may be wrong. Illustrations occur at once, such as an improper value assigned to leukocytosis or increased metabolic rate or the Wassermann test, and the grotesque, though sometimes tragic misinterpretation of roentgenograms.

3 A group diagnosis may be perfect in all its parts, statistically, physically, chemically, quantitatively it may be correct, but as a whole it is only assembled and machine-made and may be a misfit. There is lacking the important feature of personal contact between physician and patient, with proper integration and estimation of the value of details. Personal impressions count for much. It is at times more important to note how a patient tells a story than what he tells. Diagnosis is fortunately daily becoming more and more scientific but it does not yet hinge alone on hydrogen ion concentration of the blood or on the number of hours it takes a barium meal to get to the sigmoid flexure.

4 The general practitioner may be stubbornly reactionary—*laudator temporis acti*—may deny the value of the new, stick to "hunches," experience and physical examination, and thus be as prone to error as the narrowest minded specialist or the most mechanically minded technician. This is largely a defense reaction on the part of the practitioner who realizes his ignorance of much of the new knowledge. In the end it makes him a nonprogressive back number.

5 Or, with a vivid consciousness of his own limitations, distrusting his own powers, undervaluing his function as the personal adviser of the patient and as the final arbiter in the particular case, the liaison officer between patient and specialist, the general practitioner strips himself of self-respect and humbly, almost apologetically, becomes a mere distributor to others of patients who are in need of diagnosis.

What way is there out of these difficulties? How may these dangers be avoided? What is the future of the general practitioner in the matter of diagnosis? And, of greater importance, what is the future of the patient, whose welfare should be paramount? The solution of these questions is in process of evolution. I can advance only a few ideas, which are discussed more from the standpoint of the physician than of the patient, the latter really being the logical method. Time may show these ideas to be prophetic or otherwise—even foolish. There is, it seems to me, a safe and sane

middle ground that preserves the best of the features of the old time diagnosis but permits and encourages the use of the new

In the first place the general practitioner should cease apologizing. He should feel that there is a place for personality in diagnosis as in treatment. The roentgenogram may show gallstones but what those gallstones mean as to the health of the patient is a matter to be decided by the physician. What may be the effect on health of a tuberculous condition shown by roentgen examination or guinea-pig inoculation is also decided by the physician in person and not by instrumental methods. Perhaps the Wassermann reaction is four plus. What that means to the patient is not for the laboratory to decide, it is a purely clinical question. A bare diagnosis of a disease condition does not always show how disease affects the particular patient, how it disturbs his functions or even whether it is the particular disease that is producing symptoms. In other words, there must be individualization in diagnosis as in treatment. The practitioner, while properly in a state of humility before the many scientific diagnostic procedures, need not be in a state of humiliation. He may still retain self-respect as he realizes that no x-ray apparatus or other instrument is at once witness, jury and judge. He, the practitioner, still has something to say as a witness, more as a jurymen, and still more as a judge.

It must be admitted—even by the stubbornest of old style practitioners—that medicine is becoming more scientific and less empiric, and that the phenomena of health, disease, physiology and pathology are more and more to be measured by formulas of physics or chemistry. The instruments and the laboratory tests are therefore of inestimable help, even though their final status is not yet definitely settled. To deride determinations of the basal metabolic rate, white blood counts, chemical tests of the blood, Wassermann tests and blood pressure estimations because, forsooth, from thirty to fifty years ago physicians practiced medicine well without them, or because these tests have at times left one in doubt or have even misled one, is as foolish as to prefer tallow dips to electric lights because Oliver Cromwell and Abraham Lincoln had light from candles or pine knots and because, at times, something is wrong with the electric current. These things have come to stay, to be improved, some destined to be replaced by something better. They must be accepted and used, but rightly.

There is a type of physician who is skilled in physical diagnosis and who regards the x-rays, the bronchoscope, the electrocardiograph, or the sputum examination as unnecessary. He still worships at the shrine of Laënnec, Skoda, Stokes and Flint. He is a fundamentalist. He cites his own successful diagnosis of thoracic aneurysm and a small patch of tuberculosis of the lung which the roentgenologist missed, and, because of the many misinterpretations of earlier or even present-day roentgenology, condemns the whole procedure.

There is no branch of diagnosis that needs greater criticism than roentgenology. Technicians have too often gone wild in diagnosing tuberculosis of the lung, tumors of the brain, diseased appendix or peritoneal adhesions to make one very charitable toward them. But roentgenology is still in its infancy, and the roentgenologist is learning and learning fast. Even the most ardent disciple of Laënnec must admit that, with all their errors due to inexperience and the uncontrolled

enthusiasm of youth, as an aid in diagnosis the x-rays are invaluable. They are not used enough in routine work. Their revelations are often so gloriously clarifying as to cause the physician to forget his chagrin at the exposure of his carelessness or his inability to recognize a definite tuberculosis of the lung, a carcinoma of the stomach, a fracture of a vertebra, or an unsuspected gallstone. The diagnostician, whether surgeon, internist or specialist, as well as the general practitioner, must lean and lean heavily on the x-rays. There must be team work and cooperation in efforts to improve technic and the interpretation of roentgenograms.

However, the x-rays, the electrocardiograph and other instruments should not be looked on as supplanting other forms of diagnosis but as helping to sharpen the physician's wits, inciting him to elicit better histories, to understand better the significance of symptoms, and to perfect him still further in physical diagnosis. The latter is in danger of becoming a lost art through neglect on the part of students, teachers and practitioners. How often does the physician slur over an examination of the chest, lazily passing on the job of determining the size of the heart or the possible patch of consolidated lung to the x-rays. He may forget that râles and prolonged expiration, heart murmur, altered second tones and gallop rhythm are beyond detection by the x-rays. He is liable to forget, or to be ignorant of the fact, that he may be able to detect tuberculosis that the x-rays may not reveal, or to determine the size and shape of a heart that may be distorted in the silhouette on the film made by faulty technic.

On the other hand, there is a danger that younger physicians especially may be carried too far by the lure of the laboratory and instrumental side of diagnosis. Physics and chemistry are spoken of as exact sciences. The various dye tests and the tests that are concerned with the microscope, titration or the incubator seem to have an air of finality about them that attracts the undergraduate or young practitioner. They are referred to as tests or instruments of precision. He learns later that some of these tests are only approximately true—that what the eye sees as it looks through the microscope or views the roentgenogram or inspects the terminal color or sediment reaction in a special test has to be interpreted by a human brain that is here just as subject to error as when it tries to translate into terms of pathology what the eye sees, the fingers feel or the ear hears when the human body is subjected to thorough physical examination by the physician. One reason for the attraction exercised by the instrument and the laboratory is that many of these laboratory reactions and instrumental workings are complicated and but poorly understood by the average physician. So, in accordance with a phenomenon of psychology, he believes in the mysterious and the nonunderstood and underestimates the well established simple methods of age-old tradition—the history, physical examination, repeated observation and comparison with similar cases recorded in the literature or those seen in practice.

The time has not yet come to discard these methods. The careful history is still the basis of diagnosis. Repeatedly one sees the skilled physician extract the history, cross-examine the patient and get facts that settle the diagnosis or give the clue that permits the further examination to be followed up by the laboratory or instrumental method. But the value of the history, 50 per cent of the diagnosis, I may say, depends on

who gets it, how it is gotten, on the skill displayed not only in getting facts on cross-examination but in interpreting them, estimating the credibility of witnesses and judging whether the testimony is of primary or secondary importance. A good knowledge of human nature is here very helpful, as is an unbiased mind. Observation, too, is undervalued. It means looking and looking again. It means looking and seeing, looking and thinking. Unhappily, it must often be said of physicians, "Eyes have they but they see not."

Again, the general practitioner or the specialist has fallen too much into the habit of having specialists make examinations that he is fit to make himself. He can ordinarily recognize a fractured fibula, a hernia, a lipoma, a uterine fibroid. He is competent to pass on most cases of diseased tonsils. He can recognize and interpret heart murmurs. He knows what lightning pains and unsteady gait may mean and is quite competent to test the knee jerks and pupils to confirm his suspicions. He need not turn the case over to the neurologist for diagnosis. He may even be able to examine the retina or larynx and to make the lumbar puncture. And surely it is not always necessary to call in a specialist for a vaginal or rectal examination, though the frequency with which the latter is omitted by the general practitioner lends point to the *bon mot* of the French physician who said that the main function of the consultant was to examine the rectum. In the ultimate analysis, the rubber glove may be classed as an instrument of precision.

In fact, there is no one in a position so advantageous for reaching a diagnosis as the family physician. He knows the early history and he knows the constitution of the patient, which, in spite of ridicule, amounts to something. The family physician also, as has been well said, sees a moving picture of the disease from its incipience, the consultant sees only one exposure and that perhaps not characteristic.

In order that the general practitioner may be fit to make a large proportion of his own diagnoses—and he should make many more than the majority—he must not only know much about disease but also know his own limitations, the limitations of specialism and those also of particular instruments and special methods. This he has not always been capable of doing. The late Dr. Hoover once told me that he felt that the Wassermann reaction had done more harm than good owing to its unreliability and to misinterpretation by the gullible practitioner. Sir James Mackenzie petulantly said that it would have been better if the stethoscope and sphygmomanometer had never been invented. Many have expressed the opinion that the world would have been better off if roentgenograms of teeth had never been taken. These views seem to me as wrong in their extreme attitude as is the position they decry, that of implicit belief in the diagnostic acumen of the Wassermann test, the blood pressure reading, the dental roentgenogram. Because there have been misinterpretations of these tests with hastily drawn conclusions and overzealous and injudicious treatment, it does not follow that the tests ought to be discarded. The error is in physicians, who have misunderstood, have wrongly conferred an attribute of infallibility on the tests and have therefore brought them into undeserved disrepute.

If recent graduates and practitioners are to know the limitations of specializing, they should be allowed in their undergraduate days to pursue the study of some one or two subjects far beyond what is commonly outlined in the college curriculum. Standardized courses,

the teaching of the fundamentals only, "only the essentials," produces an average or mediocre student who is apt to feel that he knows more than he really does. President Wilkins of Oberlin, referring particularly to undergraduate work in the college of literature and arts, says

If the modern man is to render efficient individual service in the maintenance and development of human society, he must have a large measure of significant and ordered knowledge within some special field. College education shall, therefore, be in part general, in part special—in part extensive, in part intensive.¹

If in the medical college as in the literary college the student majors in some subject, such as pediatrics, pathology, gastro-intestinal diseases or urology, he will have learned the lesson of thoroughness. He will know how to consult literature. He will be able to judge of current medical literature and will not be too prone to believe in the truth of a statement just because it is printed. He will learn of the fallibility of human judgment, the unreliability of experience, the untrustworthiness of some so-called methods of precision. He will, on the other hand, rely on certain types of experience, will regard certain combinations of history and physical, instrumental and laboratory observations as final. In other words, he will have learned how to think for himself. He will have wisdom and will know where to go for more knowledge. He will know when to call for help in diagnosis and will be a good judge of where to go to get it.

This principle of majoring in undergraduate medicine should be carried over into the life of practice. Unless this is done the practitioner goes stale. The concentration on some subject after graduation, with the sense of mastery and power and the accompanying zeal for the new, is what will save practitioners from being mere drudges, doing hackwork only, without pleasure in their work and with no sense of progress or consciousness of superiority.

If I try to summarize this rather rambling and incomplete paper it will be seen that the thought centers about the general practitioner. He is able to diagnose the majority of commoner ailments as well as a large proportion of typically frank, rarer, serious examples of well known disease. Often, however, in order to tell what is the matter in a complicated or obscure case he must appeal for help to the clinical or laboratory specialists, who may work singly or in groups. In doing this he should realize that the specialist may be narrow minded, the technician incompetent to interpret in terms of bedside disease. The group diagnosis may be lacking in information as to the essential detail, which may be lost in the blur of a characterless composite picture.

The physician should not stubbornly refuse to accept the new nor should he be carried away by its supposed scientific accuracy. He should learn to employ the laboratory and instruments as important or, at times, indispensable aids to the older methods of history taking, physical examination and personal contact.

In order rightly to judge of the need and value of special examination in a given case, he should have had undergraduate training in more than the so-called essentials of medicine. In one or two branches his studies should have been of an intensive, concentrated character that enables him to understand his own limitations and those of others. This will make him a master in some one branch, with a self-respect that

comes from the consciousness of power, will give zest to his work, will arouse in him the desire to continue such study into his life of practice and even to do investigative work of his own. It will make him a contented, forceful progressive practitioner who can trust himself and therefore be trusted by others in that most important function of the physician the telling of what is the matter with those who are ill.

I add as a postscript a quotation from Francis Bacon. While of wider application than to the diagnostician it so aptly characterizes the mental traits such a physician should possess that it is well worth recording.

For myself, I found that I was fitted for nothing so well as for the study of Truth as having a mind nimble and versatile enough to catch the resemblances of things (which is the chief point), and at the same time steady enough to fix and distinguish their subtler differences as being gifted by nature with desire to seek patience to doubt, fondness to meditate, slowness to assert, readiness to reconsider, carefulness to dispose and set in order, and as being a man that neither affects what is new nor admires what is old and that hates every kind of imposture. So I thought my nature had a kind of familiarity and relationship with Truth.

122 South Michigan Avenue

SICKNESS IN RURAL NEW YORK

MORBIDITY SURVEY OF CERTAIN DISTRICTS IN 1927

J. V. DePORTE, PH.D.

Director, Division of Vital Statistics, New York State Department of Health

ALBANY, N. Y.

In most of the civilized countries of the world, mortality is slowly approaching an irreducible minimum. In the state of New York, for example, the death rate in 1927 was 12.3, in 1890 the rate was 21.4, the highest since 1885, when the vital statistics of the state were first compiled. Thus, in the course of thirty-seven years, the death rate dropped 43 per cent. Among the many factors responsible for this great improvement, organized public and private health activities occupy a prominent place. But will the continued and extended application of these efforts produce similar results in the course of the next two score of years? The answer is clearly and emphatically no. A reduction of 43 per cent in the present death rate would mean that only seven out of a thousand persons would die in the course of a year, and this cannot be hoped for. While even large communities may for a time record rates as low as 10 or even lower, such figures cannot persist. A death rate of 10 means an average duration of life of 100 years and in a stabilized population with a normal proportion of persons in the various age groups such a permanent rate is not possible.

The death rates in the state of New York and in this country have in the past been influenced considerably by the fact that the population contained a disproportionately large number of young persons owing to the constant influx of immigrants from the countries of the Old World. In the state of New York in the decade preceding the World War, the excess of births over deaths represented only one third of the total increment of the population. By an act of Congress in 1921 the era of free immigration came to an end. In the future the United States will therefore become more and more dependent on the natural increase for

additions to its numbers. The population will grow older, i. e., there will be in proportion more old persons and fewer young persons than in the days of unrestricted immigration, and this will exert a negative influence on the recorded death rates.

The drop in the mortality recorded in recent times has been primarily due to the prevention and control of certain diseases which formerly carried off large contingents of infants and children. At the farther end of life, the death rates have not changed materially. In fact, in certain types of mortality there has apparently been an actual increase. It is quite safe to state that most of the important diseases of childhood are theoretically preventable and that in a reasonably near future deaths from diarrhea, diphtheria, measles, scar-

SICKNESS REPORT Week ending.....102.....

The number of NEW CASES attended by me during the week ending Saturday midnight was as follows

Disease	Males	Females
Appendicitis		
Arteriosclerosis		
Cancer		
Children, diseases of (outside of reportable and those listed on this card)		
Colds		
Diabetes		
Diarrhea and enteritis (under 2 years)		
Digestive disorders, other		
Gonorrhea		
Gynecological cases		
a. Operative		
b. Non-operative		
Heart disease		
Neuroses		
Nephritis, acute		
Nephritis, chronic (Bright's disease)		
Rheumatism		
a. Acute rheumatic fever		
b. Chronic arthritis		
Surgical cases		
operative, traumatic (except gynecological)		
Syphilis		
Tonsillitis		

(Signed).....M. D.

P. O.

IMPORTANT Please mail report IMMEDIATELY even if NO NEW CASES of above named diseases were attended.

Fig. 1—Report form used in morbidity survey

let fever and whooping cough will join smallpox and yellow fever in the limbo of painful memories. But the reduced volume of deaths in early ages means of necessity more deaths in middle life and old age. The abolition of physical death or even a serious effort for a marked prolongation of individual life is outside the realm of practical contemplation. If public health activities were multiplied a hundredfold, the effect on the death rate would be relatively small. What, then, is public health work to look to? Here I can think of no finer ideal than Metchnikoff's vision of a life free from bodily ailments and "after life's fitful fever" a painless descent into the sleep eternal.

From the point of view of society as a whole, sickness is more important than death. Popular experience has wisely coupled the terms health and happiness.

1. Quoted in Francis Bacon, "Of the Study of Truth," London, 1612.

Health is the basic element in the life of any human group. Individuals may and have reached the heights of achievement even with a handicap of indifferent or poor health, but no social unit can function normally and preserve and develop its cultural and material treasure unless a sufficiently large proportion of its members possess the essential factor of physical health. Public health work is successful so far as it helps to bring about a higher degree of healthfulness in a community. To guide these activities and to measure their effect there must be, first of all, accurate information regarding the prevalence of ill health.

In New York and in most of the other states, the reporting by physicians of certain types of communicable disease is compulsory. These diseases represent, however, only a small fraction of all causes of sickness. The need of more complete morbidity statistics comprising other than the reportable diseases was recognized long ago. The pioneer work in this field was done by Dr. John S. Billings, author of the morbidity tables of the 1880 federal census,¹ the purpose of which was to determine "the number of [persons] who were on the first of June [1880] so sick or disabled as to be unable to pursue their ordinary occupations." Similar tables were compiled in connection with the census of 1890. Then there are "the records of a few fraternal societies, of some industrial establishments, benefit funds, the community sickness censuses of Messrs. Frankel and Dublin,² some data from casualty companies writing health business and a few tables of acute and chronic diseases published by the Commonwealth of Massachusetts."³ Among later studies may be mentioned the survey of sickness in Dutchess County, N. Y.,⁴ "the illness census" taken in certain districts in New York City in 1915 and 1917,⁵ the sickness census of the Framingham demonstration,⁶ and the studies of morbidity in Hagerstown, Md., conducted by the U. S. Public Health Service from 1921 to 1924.⁷ These several investigations, differing in many respects, have in common the method of procedure: the information in all cases was obtained by the census method, directly from the persons residing in the census area, the facts in some cases being compared with the records of physicians.

GENERAL PLAN OF SURVEY

Several years ago it occurred to us in the New York State Department of Health to attempt to obtain these statistics through direct reporting by the attending physicians. In 1925 I⁸ outlined the project as follows:

We usually know what disease a patient had when he died, but we do not know what other diseases in the past had prepared the ground for the fatal attack. The entire group of diseases that are at present reportable accounts for only one

quarter of all deaths, and not even all of these causes are reported completely. For instance, it is agreed that for every death from tuberculosis there are from seven to nine active cases of the disease, and yet in the entire state the number of cases reported last year was only double the number of deaths, and several counties registered more deaths than cases. Regarding the prevalence of diseases upon which no reports are required, we know very little. Here our figures are mere guesses, based on scattering data from hospitals and sanatoria, insurance companies, schools.

We must have facts, not conjectures. The Division of Vital Statistics is now endeavoring to work out a plan for the collection of morbidity statistics, the success of which will depend entirely upon the measure of assistance which you gentlemen will find possible to give. Details of this scheme will be communicated to you as soon as they are perfected.

SUGGESTIONS FOR REPORTING		
In using the form on the reverse side of this card, please follow the directions carefully.		
Use the card daily, putting a mark for each new case. Thus:		
Disease	Males	Females
Tonsillitis	///	//
And as further new cases are seen, simply add to the check marks, thus:		
	////	////
The value of this record lies in its completeness, it is therefore important to send in the card weekly, even when NO new cases of the specified disease are seen.		
Under "Colds" enter bronchitis, coryza, "grippe", laryngitis, pharyngitis and similar conditions.		
Under "Children, diseases of" enter all forms that are at present not reportable or shown elsewhere on the card for instance rickets, pyelitis, malnutrition, etc.		
Under "Neuroses" include conditions such as 'worry', "indigestion", "neuritis", any condition that is in your opinion based on a functional disturbance of the nervous system.		
Under "Surgical" enter operations for any cause injuries due to accidents, etc.		
3-1 2-5000 (11-1921)		

Fig. 2—Reverse of report form used in survey.

In coming to you with a request for cooperation I am moved by the conviction that most of us have an urge to spend some of our time in doing what we feel is worth while and for which we can expect no compensation other than the approval of our inner selves and the possible approbation of our neighbors. You will agree that the greatest need of public health work is accurate information regarding the prevalence of disease, without which a continuous constructive policy of prevention is difficult, if not impossible. It is in this field, in my opinion, that the individual health officer can make his greatest contribution to the happiness of his fellows, and it is in this field that public health work must have the guidance of vital statistics.

The plan lay dormant for more than a year when, with the cooperation of the district state health officers, it was once more brought to the attention of practicing physicians. By this time we had decided to limit ourselves to a twelve months' study in the rural part of

1 Tenth Census of the United States volume 12 1880

2 Frankel L. K. and Dublin L. I. Some Recent Morbidity Data. A Summary of Seven Community Sickness Surveys Made Among Policy Holders of the Metropolitan Life Insurance Company 1915 to 1917 compiled by Margaret L. Stecker 1919

3 Kopf E. W. Some Essentials of Sickness Statistics. Proc. Casualty Actuarial and Statistical Society of America volume 4 part 1 number 9

4 State Charities Aid Association. Sickness in Dutchess County N. Y. September 1915

5 Guilfoyle W. H. Illness Census Taken in Health District No. 1. Monthly Bulletin of the Department of Health of the City of New York March 1916 Wynne S. W. Second Illness Census in the Experimental Health District. Ibid. November 1916 Illness Census. Ibid. January 1918

6 The Sickness Census. Framingham Monograph 2. Framingham Community Health and Tuberculosis Demonstration of the National Tuberculosis Association June 1918

7 Sydenstricker Edgar. Hagerstown Morbidity Studies. Pub. Health Rep. 41. 2069 (Sept. 24) 2186 (Oct. 8) 1926 42:121 (Jan. 14) 1565 (June 10) 1689 (June 24) 1927

8 DePorte J. V. Read before the Annual Conference of Health Officers at Saratoga Springs June 23 1925

the state and, so far as possible, to endeavor to secure the cooperation of all physicians in well defined small areas. The results of this appeal were most gratifying. By the end of 1926 we had gained the active interest of 150 physicians, and the survey commenced the first week of January, 1927.

THE SICKNESS REPORT FORM

The report form used in the survey (fig. 1) was, of course, far from perfect. We were guided in its preparation by two basic considerations—brevity and

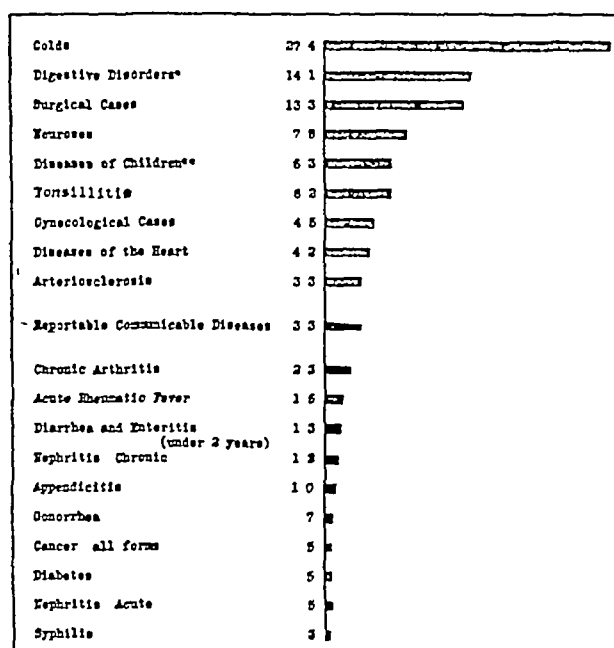


Fig. 3—Percentage distribution of all cases of sickness. *except diarrhea and enteritis under 2 years of age, **except communicable or others shown in this diagram.

simplicity. The list of causes had to be brief, since we could not ask a busy rural practitioner to give the time that would be necessary to fill out a blank containing all of even the more important types of sickness. For the same reason we limited the subdivisions to sex only, making no provision for age, occupation and other facts relating to the personal history of the patient or for the duration and degree of severity of the illness. One proposed and very desirable heading, "all other types of diseases" which would have given us a picture of the entire morbidity situation, had to be omitted because some of the physicians preferred not to disclose the general extent of their practice.

The physicians were asked to report only new cases of the various types of sickness indicated on the list and not new patients. In other words, if John Smith called on a physician and the latter diagnosed his condition as an ordinary cold, he entered on the card, in the column of males, one case of cold. If a few weeks later the physician found that the same John Smith suffered an attack of appendicitis, he entered on the card one case of appendicitis. The several explicit rubrics were to include all types of disease outlined under the corresponding heads in the International List of Causes of Death. Thus, "cancer" was to comprise numbers 43-49 of the international list, "heart disease," numbers 87-90, and so on. The term "children" related to persons under 16 years of age. Explanation of the other multifarious titles, together with certain sugges-

tions, was given on the reverse of the card (fig. 2). The physicians were furnished stamped and addressed envelopes in which to mail the cards to the division of vital statistics each week.

Our list included gonorrhea and syphilis because reports on these diseases are ordinarily secured through laboratories and not directly from physicians. In addition to the types of sickness shown on the report form, we also tabulated the cases of sickness reported by the participating physicians to the division of communicable diseases. Whenever a report contained an ambiguous or contradictory entry, or if a physician was in doubt about reporting or not reporting a case in his practice, the necessary adjustments were made, either by correspondence or through the assistance of the district state health officers.

The districts represented in the study were scattered throughout thirty-three counties, the largest village having a population of almost 6,000, the others being small villages or townships not containing incorporated places of any size.

In the course of the year forty-three physicians were obliged, for one reason or another, to discontinue their reports. We have, therefore, complete returns for the entire fifty-two weeks of the year 1927 from 107 physicians. The population served by these physicians, according to the best available information, numbered about 101,000, or 944 persons for each physician. The average number of persons to each physician in the entire state of New York, exclusive of New York City, Jan. 1, 1928, was 841. Since in rural districts the ratio is higher than in cities, our estimate appears quite reasonable.

GENERAL RESULTS

A summary of our results is presented in table 1. The cases of sickness totaled 98,069. It is possible that this figure contains a number of repetitions, in other words, that some patients were treated for the same condition by more than one physician. The practice of changing physicians is, however, not common in rural territory and the element of error introduced by it is probably inconsiderable. The total of recorded cases was, roughly, equal to our estimated population, the cases, therefore, averaged one for each person annually. The fact that our estimated population is practically

TABLE 1—Cases of All Reportable and Nonreportable Diseases, by Sex

	Total		Male		Female	
	Num ber	Per Cent	Num ber	Per Cent	Num ber	Per Cent
Total	98,069	100.0	46,090	100.0	51,979	100.0
Nonreportable diseases	91,537	93.4	41,473	90.2	50,064	96.3
Reportable communicable diseases	6,532	6.6	4,617	9.8	1,915	3.7

100,000 permits us to interpret the figures shown in our detailed tables in terms of rates. In other words, if the cases of a certain disease numbered 200, the rate of sickness from that disease may be taken, roughly, as 200 per hundred thousand of population.

The total number of cases of sickness reported among women exceeded the total for men by 13 per cent. While this fact, in general, corresponds to the common observation of the greater prevalence of sickness among women, we cannot say anything about the indicated disparity without definite knowledge of the relative numbers of men and women in our territory, since a portion of the excess may have been due to a greater proportion of women in the population.

Cases of communicable diseases that are reportable under the provisions of the New York State Sanitary Code numbered 3,212, or 33 per cent of the total cases of all types. The proportion among males was 35 per cent, among females it was somewhat lower, 31 per cent.

Figure 3 shows the relative prevalence of the reportable communicable diseases as compared with the specified types of the nonreportable diseases. Here it may be observed that cases of nine groups of the latter were more prevalent than the sum of all reportable communicable diseases.

NONREPORTABLE DISEASES

Table 2 shows in detail the distribution of the nonreportable diseases.

The leading cause was colds, with a total of almost 27,000 cases, or 28.3 per cent of all cases. Digestive disorders were responsible for 14.6 per cent, followed by all types of surgical cases (except gynecologic), 13.7 per cent. Thus, more than one half (56.6 per cent) of all causes of sickness were listed under these three heads.

The proportions which the various types of sickness formed of the total number of cases reported among males and females were, in most cases, dissimilar. The differences were especially marked under surgical cases, which represented one fifth of all cases among males as compared with only 8.5 per cent among females, cases of neuroses (this term in our survey comprised all types of sickness that were in the opinion of the attending physician based on a functional disturbance of the nervous system) were twice as prevalent among women as compared with the ratio among men (10.4 and 5.4 per cent, respectively).

TABLE 2—Cases and Percentage Distribution of Nonreportable Diseases, by Sex

Cause of Sickness	Total		Male		Female	
	Num ber	Per Cent	Num ber	Per Cent	Num ber	Per Cent
All nonreportable diseases	94,657	100.0	44,478	100.0	50,379	100.0
Colds	26,558	28.3	13,265	29.8	13,593	27.0
Digestive disorders (other than diarrhea and enteritis under 2 years)	13,625	14.6	6,254	14.1	7,571	15.0
Surgical cases—operative, traumatic (except gynecologic)	13,031	13.7	8,761	19.7	4,270	8.5
Neuroses	7,635	8.0	2,302	5.4	5,243	10.4
Diseases of children (except communicable and those listed in this table)	6,178	6.5	2,074	4.7	3,109	6.3
Tonsillitis	6,038	6.4	2,017	4.5	3,121	6.2
Gynecologic cases	4,422	4.7			4,422	8.8
Operative	761	0.8			761	1.5
Nonoperative	3,671	3.9			3,671	7.3
Heart disease	4,123	4.3	1,051	2.4	2,172	4.3
Arteriosclerosis	3,234	3.4	1,352	3.0	1,882	3.7
Chronic arthritis	2,240	2.4	1,069	2.5	1,148	2.3
Acute rheumatic fever	1,543	1.6	789	1.8	764	1.5
Diarrhea and enteritis (under 2 years)	1,308	1.4	640	1.4	668	1.3
Chronic interstitial nephritis (Bright's disease)	1,134	1.2	482	1.1	652	1.3
Appendicitis	960	1.0	453	1.0	513	1.0
Gonorrhea	644	0.7	521	1.2	123	0.2
Diabetes	565	0.6	173	0.4	392	0.7
Cancer (all forms)	464	0.5	156	0.4	308	0.6
Acute nephritis	450	0.5	163	0.4	287	0.6
Syphilis	264	0.3	141	0.3	123	0.2

The number of cases of gonorrhea among men (521) was more than four times that among women (123), the corresponding percentages being 1.2 and 0.2. In considering these figures, it must be remembered, however, that many cases of gonorrhea among women were undoubtedly listed under the head of gynecologic conditions.

REPORTABLE DISEASES

Table 3 lists the communicable diseases reported by the cooperating physicians.

The leading disease was measles, with 37.4 per cent of all cases followed by chickenpox, 14 per cent, and whooping cough, 13.4 per cent. If to this list are added

TABLE 3—Cases and Percentage Distribution of Reportable Communicable Diseases, by Sex

Cause of Sickness	Total		Male		Female	
	Num ber	Per Cent	Num ber	Per Cent	Num ber	Per Cent
All reportable communicable diseases	3,212	100.0	1,618	100.0	1,594	100.0
Measles	1,231	37.4	607	37.5	594	37.3
Chickenpox	460	14.0	224	13.8	226	14.2
Whooping cough	432	13.4	221	13.7	211	13.2
Scarlet fever	306	9.5	140	8.7	166	10.4
Pneumonia (all forms)	200	6.1	133	8.5	122	7.7
Broncho or lobular	110	3.7	55	3.4	64	4.0
Acute lobar	131	4.1	78	4.8	53	3.3
Unspecified	10	0.3	5	0.3	5	0.3
Mumps	222	6.9	127	7.8	95	6.0
German measles	114	3.5	45	2.8	69	4.3
Tuberculosis (all forms)...	100	3.1	47	2.9	53	3.3
Pulmonary and acute						
miliary	80	2.5	32	2.0	48	3.0
Other forms	20	0.6	15	0.9	5	0.3
Diphtheria (membranous croup)	49	1.5	26	1.6	23	1.4
Poliomyelitis acute anterior (infantile paralysis)	18	0.6	11	0.7	7	0.4
Smallpox	16	0.5	10	0.6	6	0.4
Erysipelas	14	0.4	8	0.5	6	0.4
Vaccinia angina...	10	0.3	5	0.3	5	0.3
Epidemic or streptococcal (septic) sore throat	0	0.3	5	0.3	4	0.3
Typhoid (except paratyphoid)	7	0.2	3	0.2	4	0.3
Epidemic encephalitis	3	0.1	1	0.1	2	0.1
Epidemic cerebrospinal meningitis	1	—*	—	—	1	0.1

* Less than 1 per cent.

syphilis and gonorrhea, which are not reportable, it is found that, next to measles, gonorrhea was the most prevalent communicable disease in the rural territory represented in the survey. Syphilis falls into sixth place, taking precedence over pneumonia and tuberculosis.

The percentage distribution of the several causes of sickness among males and females was quite similar. Cases of scarlet fever, German measles and tuberculosis were somewhat more numerous among females, while among males there was relatively a greater number of cases of whooping cough, mumps and pneumonia.

Seasonal Distribution of Morbidity—When the returns for the four quarters of the year are expressed in terms of the total number of cases reported for the entire twelve month period, it is found that the greatest amount of sickness, 28.6 per cent, occurred during the three winter months and the least amount, 22.9 per cent, during the third quarter. The distribution for the two sexes differed slightly among females the maximum during the first quarter and the minimum during the third quarter were somewhat more accentuated than among males.

The peak of cases of nonreportable diseases was recorded during the first quarter of the year, almost entirely because of the greater prevalence of colds. Among the individual causes we note that almost two fifths of the cases of colds were recorded during the first quarter, while during the third quarter these cases were only 15 per cent of the total for the year. The distribution of cases of tonsillitis was similar to that of colds, although the peak was not accentuated to the same degree, the maximum during the first quarter representing 31.9 per cent and the minimum during the third quarter 20.1 per cent of all cases. Of the outstanding differences in the seasonal distribution of the

other groups of causes, the following may be mentioned. In the group of children's diseases, other than reportable and those listed on the form, the peak, 27 per cent, was recorded during the first quarter of the year and the minimum, 22.8 per cent, during the second quarter. Diarrhea and enteritis, under 2 years, had a peak during the third quarter of 43.6, and a minimum during the first two quarters of 15.5 and 15.6 per cent, respectively.

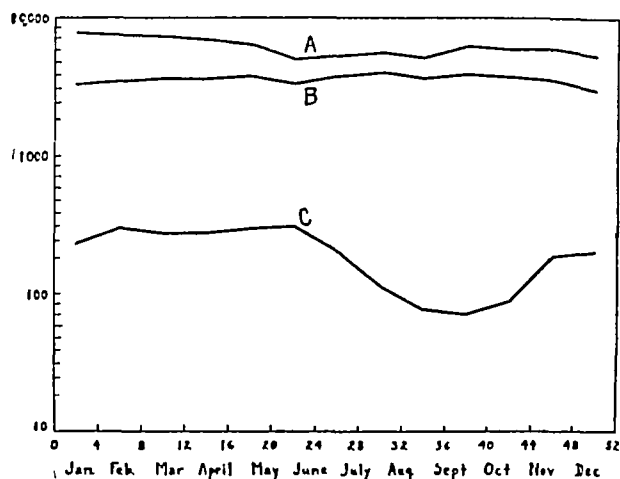


Fig. 4—Cases of all reportable and nonreportable diseases by four week periods. A, all nonreportable diseases; B, all nonreportable diseases except colds, diarrhea and enteritis (under 2 years) and tonsillitis; C, all reportable communicable diseases.

The seasonal distribution of all other digestive disorders was similar to that of diarrhea, except that the high and low points differed less than in the former group, the peak, 27.2 per cent, being recorded in the third quarter and the minimum, 23.8, in the second quarter. The group of surgical cases showed a marked peak in the third quarter, 31.7, and a minimum in the first quarter, 19.2. This group embraces all cases in which surgical treatment was required, and the peak in the summer months undoubtedly reflects the increased number of

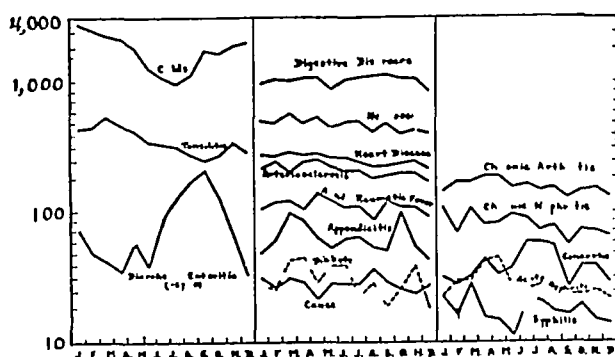


Fig. 5—Cases of specified nonreportable diseases by four week periods except diarrhea and enteritis under 2 years.

accidents during that period. Gonorrhea had a peak in the summer months of 31.4 per cent, and a low point of 20.2 per cent in the first quarter. In the group of gynecologic cases containing undoubtedly a large proportion of cases of gonorrhea, there was also reported a peak in the third quarter of the year of 27.2 per cent, with a minimum of 23.6 per cent during the second quarter.

The seasonal variation of the entire group of reportable communicable diseases was strikingly more pro-

nounced than that of the nonreportable group. More than one third of all cases, or 37.5 per cent, were recorded during the second quarter, while the minimum in the summer months was less than a third of this proportion, 11.3 per cent.

To analyze the seasonal changes in greater detail we have plotted the percentage distribution of cases of the nonreportable and of the reportable diseases by four-week periods. Curve A in figure 4 shows the seasonal variations of the entire group of the nonreportable diseases, the cases numbering 94,857. Here a peak was noted during the first four-week period, the month of January, when the number of cases represented 9 per cent of the annual total. In the following four-week periods the relative number of cases declined, reaching a minimum of 6.6 per cent during the sixth four-week period (May 22-June 18).

When the important component parts of this large group of cases are considered, there is first of all, the heterogeneous group of "colds" representing 28.3 per

TABLE 4—Percentage Distribution of Cases of Nonreportable and of All Reportable Communicable Diseases by Quarters of the Year

Cause of Sickness	Total			
	First Quarter	Second Quarter	Third Quarter	Fourth Quarter
All nonreportable diseases	28.6	21.2	23.3	21.1
Colds	37.5	21.8	15.3	25.1
Digestive disorders (other than diarrhea and enteritis under 2 years)	21.4	23.8	27.2	21.6
Surgical cases—operative traumatic (except gynecologic)	19.2	24.5	31.7	24.6
Neuroses	26.9	25.0	24.8	22.4
Diseases of children (except communicable and those listed in this table)	21.6	22.8	25.6	27.0
Tonsillitis	31.9	27.5	20.1	20.5
Gynecologic cases	25.9	23.6	27.2	24.3
Operative	33.1	25.6	29.9	19.4
Nonoperative	21.3	23.2	27.2	25.3
Heart disease	27.8	26.0	22.9	27.3
Arterio sclerosis	27.0	29.2	22.3	21.6
Chronic arthritis	26.0	24.1	23.1	22.8
Acute rheumatic fever	20.3	25.1	21.3	23.3
Diarrhea and enteritis (under 2 years)	15.5	15.6	43.6	25.3
Chronic interstitial nephritis (Bright's disease)	28.3	24.8	23.5	22.4
Appendicitis	29.7	25.0	22.9	24.8
Gonorrhea	20.2	29.1	31.4	22.4
Diabetes	29.3	25.3	19.2	22.2
Cancer (all forms)	29.6	23.1	27.8	22.6
Acute nephritis	26.0	22.2	21.1	20.7
Syphilis	32.9	21.6	21.2	22.0
All reportable communicable diseases	33.6	37.5	11.3	17.7

cent of all the nonreportable diseases. This group has a very marked seasonal variation (fig. 5), the maximum proportion of cases (13.3 per cent) being reported during the first four-week period and the minimum (3.7) in the eighth four-week period (July 17-August 13). Two other types of sickness exhibit definite seasonal changes—tonsillitis and diarrhea and enteritis under 2 years (fig. 5). The curve for tonsillitis is quite like that of colds, while the curve for diarrhea and enteritis shows a peak during the summer months with low points in the fall and winter. When the cases of the three causes mentioned are subtracted from the total, the residual cases do not exhibit any definite trend (fig. 4 B).

The group of reportable diseases has a definite seasonal trend, with high points in the late fall, winter and early spring, and a trough in the summer and early fall (fig. 4 C).

The curves of the individual important groups of nonreportable diseases other than those mentioned earlier do not exhibit any marked seasonal changes (fig. 5). It is interesting to note the similarity in the curves for heart disease and arteriosclerosis. This is

very likely due to the occasional difficulty of placing the diagnosis under one or the other of these two heads, in other words, some conditions entered under the rubric arteriosclerosis could probably, with equal justice, be diagnosed as a type of heart disease.

Figure 6 pictures the seasonal variations of the several individual groups of reportable communicable diseases. They all have a definite maximum point in the winter months, with the exception of whooping cough, in which a second peak occurred in the summer.

Morbidity in Villages and Towns—We have tabulated our results under the heads of "villages" and "towns," the former term including incorporated places of any size, the latter referring to the unincorporated territory. The population of the villages ranged from 125 to 5,907. The cases in the group of villages numbered 82,518 and were reported by eighty-three physicians, averaging 994 cases reported by a physician. In the rest of the rural territory 15,551 cases were reported by twenty-four physicians, or an average of 648 cases for one physician. In the entire area represented in our survey, the cases averaged 917 for a physician. Table 5 shows the number of cases and the percentage distribution according to sex in these two divisions, villages and towns. The percentage distribution in general showed no material differences, with the following exceptions: reportable communicable diseases represented 34 per cent of all cases in the villages as compared with only 26 per cent in the towns. Among the

The percentages of morbidity and mortality from the specified causes are given in table 6. Colds, including tonsillitis, were the leading cause of sickness, with 57.3 per cent of all cases of the abridged group of diseases, the mortality from this cause represented only 3.2 per cent of the total. The entire group of the reportable communicable diseases was responsible for 56 per cent

TABLE 5—Number and Percentage Distribution of Cases of Nonreportable and of All Reportable Communicable Diseases by Villages and Towns

Cause of Sickness	Villages		Towns	
	Num-ber	Per Cent	Num-ber	Per Cent
All causes included in morbidity survey	82,518	100.0	15,551	100.0
All nonreportable diseases	79,705	93.6	15,152	97.4
Colds	27,593	27.4	4,263	27.4
Digestive disorders	11,503	14.3	2,022	13.0
Surgical cases (except gynecologic)	10,673	12.9	2,833	15.1
Neuroses	6,400	7.8	1,175	7.0
Diseases of children (except communicable and those listed in this table)	5,215	6.4	928	6.0
Tonsillitis	4,634	6.0	1,084	7.0
Gynecologic cases	3,752	4.5	670	4.3
Operative	645	0.7	206	1.3
Nonoperative	3,207	3.9	464	3.0
Diseases of the heart	3,453	4.2	670	4.3
Arteriosclerosis	2,745	3.3	489	3.1
Chronic arthritis	1,934	2.3	306	2.0
Acute rheumatic fever	1,305	1.6	238	1.5
Diarrhea and enteritis (under 2 years)	1,123	1.4	180	1.2
Chronic nephritis	678	0.8	155	1.0
Appendicitis	791	1.0	175	1.1
Gonorrhea	500	0.7	84	0.5
Cancer (all forms)	303	0.5	69	0.4
Diabetes	441	0.5	64	0.4
Acute nephritis	307	0.4	83	0.5
Syphilis	221	0.3	43	0.3
All reportable communicable diseases	2,813	3.4	899	2.6

of all cases of sickness and four times that proportion (22.4 per cent) of deaths. Somewhat more than four fifths (81.2 per cent) of all deaths shown in the table were due to the five leading causes of death—diseases of the heart, cancer, chronic nephritis, pneumonia and

TABLE 6—Percentage Distribution of Cases in Morbidity Survey and of Mortality in the State, Exclusive of New York City, from Specified Nonreportable and from All Reportable Communicable Diseases

Causes of Sickness and Death	Percentage of Cases Reported in Morbidity Survey*	Percentage of Deaths in the State Exclusive of New York City
All causes included in morbidity survey*	100.0	100.0
All nonreportable diseases	94.4	77.6
Colds including tonsillitis	57.3	3.2
Gynecologic cases	7.7	0.5
Heart disease	7.2	34.0
Arteriosclerosis	5.6	4.3
Chronic arthritis	3.9	0.2
Acute rheumatic fever	2.7	0.3
Diarrhea and enteritis (under 2 years)	2.3	1.6
Chronic interstitial nephritis (Bright's disease)	2.0	13.0
Appendicitis	1.7	1.5
Gonorrhea	1.1	
Diabetes	0.9	2.9
Cancer (all forms)	0.8	14.7
Acute nephritis	0.8	0.6
Syphilis	0.5	0.7
All reported communicable diseases	5.6	22.4

* Exclusive of diseases of children: digestive disorders (other than diarrhea and enteritis under 2 years), neuroses and surgical cases.

tuberculosis, while the corresponding cases of sickness represented barely one tenth (10.7 per cent) of the total.

Although the territory from which the mortality figures were drawn is greater in size and more diverse in character than the rural area of our survey, the main facts brought out in the preceding paragraph are

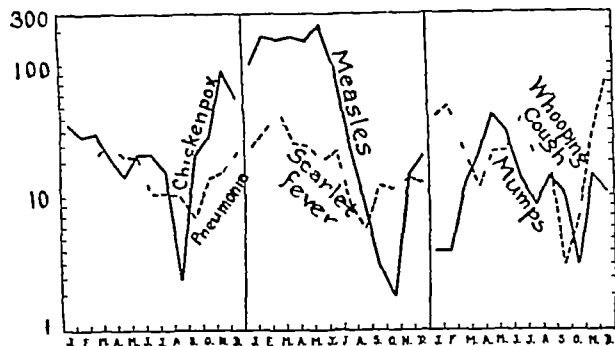


Fig. 6—Cases of specified reportable diseases by four week periods

nonreportable diseases it is noted that the towns had a larger proportion of surgical cases (15.1 per cent, the villages, 12.9) and a smaller proportion of cases of digestive disorders (13.0 as compared with 14.3 per cent).

Percentage Distribution of Morbidity and Mortality by Cause—An interesting comparison is that of the relative morbidity and mortality from the various causes. Here we are obliged to employ the mortality figures for the entire state, exclusive of New York City, for the year of the survey, 1927, because unfortunately the mortality experience of the population represented in our survey was not available.

Since several of the groups of causes were rather too comprehensive, the mortality caused by these diseases could not be evaluated. These groups were diseases of children (outside of reportable and those listed on the report form), digestive disorders (other than diarrhea and enteritis under 2 years), neuroses, and surgical conditions. Deaths in the upstate territory ascribed to all other types of sickness listed on the report card, and also to the entire group of reportable communicable diseases, numbered 46,349, representing 65.7 per cent of the deaths from all causes.

undoubtedly true also for the more limited region. We are therefore on solid ground in stating that the diseases responsible for the bulk of mortality play a comparatively inconspicuous role among the causes of prevailing ill health.

CONCLUSIONS

The morbidity survey of a considerable section of rural New York has accomplished I believe two useful purposes. It has given us an indication of the absolute and relative prevalence of certain important causes of illness, regarding which our knowledge is quite scanty. The survey has also demonstrated the numerically unimportant position of the reportable communicable diseases. Even if we were to add the nonreportable diseases which come within the field of organized public health activities, as diarrhea and enteritis (under 2 years), syphilis and gonorrhea the cases of the enlarged group of diseases would equal only 56 per cent of all causes of illness represented in the survey.

The function of public and private health agencies is the control and prevention of sickness. The experiment made by the New York State Department of Health to secure sickness statistics for a large general group of the population through direct reporting by the attending physicians leads us to conclude that greater attention should be given by public health authorities and the practicing medical profession to the nonreportable diseases which are responsible for most of the prevailing ill health.

A SURVEY OF AMEBIC DYSENTERY IN CHICAGO*

CHARLES SPENCER WILLIAMSON, M.D.

BERTHA KAPLAN, M.S.
CHICAGO

AND

J. C. GEIGER, M.D.
SAN FRANCISCO

INTRODUCTION

By CHARLES SPENCER WILLIAMSON

The stimulus for the survey of which these reports are a summary lay in the fact that I had occasion to see in private practice within a very short time several cases of amebic dysentery occurring under rather unusual conditions.

The first patient had a very large hepatic abscess, which had been opened and drained, and the patient was steadily going down hill. It was stated that examinations of the pus had been made with negative results. I saw the patient after the abscess had been draining for a long time, and on a personal examination of the pus, which was of the typical anchovy sauce type, found typical endamebas in large numbers. Vigorous treatment with emetine caused a remarkable clearing up of the entire process in a short time. The patient was living in a downtown hotel.

A short time afterward another patient was seen, a middle aged man, living in the same hotel. This patient had a peculiar mass in the right quadrant, the nature of which was doubtful, but as the patient had a severe diarrhea, a stool examination was made, which disclosed myriads of amebas. The patient was immediately taken to the hospital and operated on. A large retrocecal abscess due to a perforation of the bowel was found, and in the pus from the abscess amebas were abundant.

The third patient was an Italian, who had, however, been in this country for many years and had always worked at the same job. His work was in the same building in which the hotel was located. It is interesting to note that this patient had been treated several times in a well known hospital for colitis, and a fatal prognosis had been made. The stools were of the typical bloody, mucoid type, and amebas were present in enormous numbers. In spite of the long duration of his illness a perfect recovery was made under treatment with emetine.

The fourth patient was not seen by me, but in describing the other three cases to a colleague in pathology, I learned that another case had occurred in the hotel next door to the one mentioned, and that the patient had died without a diagnosis, the autopsy showing a fuldroyant amebic dysentery.

The fifth patient was a traveling salesman, who spent a couple of months of each year at the hotel in question and when first seen had been residing there for several weeks.

At this point it could hardly be doubted that it was not merely fortuitous that these cases of dysentery should appear in such quick succession in one neighborhood, and a carrier among the food handlers seemed the most plausible explanation.

A meeting was arranged with the owners of the hotel, and when the matter was laid before them they responded quickly and heartily to the suggestion that the parasitologist of the Research Hospital of the University of Illinois make a careful survey of all the food handlers and kitchen help. This was accordingly undertaken by one of us (B. K.) and very shortly an active case of the disease was found among the kitchen help. At this time we requested a consultation with the health commissioner who cooperated in every possible way and detailed one of us (J. C. G.), then assistant commissioner, to investigate the entire matter from the epidemiologic standpoint.

Following numerous consultations a study of the food handlers and kitchen help of a number of the more important Chicago hotels was undertaken. Before these studies were very far under way three additional private cases were seen by me, but in these three no connection with the previous cases could be made out. A detailed report of these investigations follows, together with a study of the incidence of endamebas in the population of Chicago at large.

In order to insure appropriate treatment for the carriers who were found, the commissioner ordered all such patients to the Research Hospital, where they were kept until freed from infestation.

The treatment was as follows: Emetine bismuth iodide, 3 grains (0.2 Gm.) daily, 1 grain (0.065 Gm.) being given after each meal, and emetine hydrochloride, one-half grain (0.03 Gm.) twice daily, hypodermically, for ten days. In some cases powdered ipecac was given through the duodenal tube. Soap suds enemata were followed by quinine irrigations (1:5000 solution, gradually increased to 1:1000) daily.

METHOD OF STUDY

By BERTHA KAPLAN

The examinations were carried out at the hotel. A room with a bath was obtained, and specimens were collected (by giving a glycerin suppository whenever necessary) in paraffined containers of 60 cc. capacity and examined immediately, one person being taken at a time. The greatest care was exercised in making the

* From the Department of Medicine, University of Illinois College of Medicine and the Department of Health, City of Chicago.

microscopic examinations for worms, ova and intestinal protozoa, with a special view to finding *Endameba histolytica* cysts as well as living forms. The work was done on the basis of two examinations per capita. All smears were made of the proper opacity ("reading opacity"), warm water being used for some smears, iodine (5 per cent aqueous solution of potassium iodide saturated with iodine and diluted with equal parts of distilled water before using) for others, and eosin (1 1,000 aqueous) preparations. The water preparations showed living forms as well as encysted protozoa, ova and worms. In the eosin preparations the living as well as the encysted forms remained as unstained refractile bodies, while everything else including the dead cysts stained pink. The iodine stained the encysted forms, making possible cytologic study.

This survey at the place where the cases of amebic dysentery occurred resulted in the uncovering of one case and two carriers of *Endameba histolytica*. Of the carriers showing encysted *Endameba histolytica*, one was a pantry girl and the other a vegetable girl. The clinical case occurred in a night cook (Assyrian) who had been ill with amebic dysentery for a number of weeks and had been treated by an Assyrian doctor who failed to report the case to the health department. This cook, being on night duty, when the work is not very heavy, was able to pursue his duties most of the time, having stayed out only two or three days with the excuse that he was suffering with "stomach trouble."

The history obtained from this cook was not entirely satisfactory, he failed to give a history of having had amebic dysentery before but stated that he had always been in rather poor health. It is not possible to state definitely which of these three persons was the source of infection. Our own experiments and those of the English workers Walker and Sellards and of Dobell have shown that the living forms are not infectious; the living forms produce the clinical symptoms of amebic dysentery, and the encysted forms transmit the disease. Whether this patient had been a healthy carrier prior to developing the clinical symptoms, or whether he as well as the others with clinical amebic dysentery were victims of one or both of the carriers of encysted *Endameba histolytica* cannot be ascertained.

O'Conner and Wenyon in their work in Egypt found the largest number of carriers of amebic dysentery among the food handlers and prisoners. This may be explained on the basis of insanitary conditions of the soil as well as of the institutions. Food handlers working with vegetables coming from contaminated soil become infected, after which dissemination is very easy by careless workers and by means of flies. With food handlers in this country conditions are different. Most, if not all, of the vegetables come from soil free from amebas, but, should there be even one carrier in a kitchen this furnishes a ready means of disseminating the disease.

Most of the patients at Cook County Hospital gave a history of having worked on the railroad in the North or in the South and of having used stagnant water for drinking purposes, two of the patients had worked at the stockyards and two were ex-sailors, one coming from Egypt and the other having been in India. Some of the patients came from the South, and several stated that they had never been outside the city.

Among the 1,148 food handlers the protozoan infections were distributed as shown in table 1.

In these 1,148 persons, metazoan infections were found as follows. Seven showed ova of *Trichuris*

trichiura, three showed ova and segments of *Taenia saginata*, three showed ova of *Necator americanus* (one, a Mexican, having been here only six months, and two Filipinos), one showed *Ascaris lumbricoides* ova and another showed *Strongyloides stercoralis* in large numbers.

The number of flagellates, worms and ova might have been increased by administering salts, because most of the specimens obtained were from the lower bowel, the region of the ameba, whereas most worms and the flagellates are found in the small intestine. The number of specimens showing ova and worms might also have been enhanced by using concentration methods, but that was not convenient and since the important point at issue was the uncovering of cases of amebic infestation and carriers we were not so much concerned with the others.

The distribution of the carriers and cases of *Endameba histolytica* was as follows. The active cases were found in a night cook at the place where the epidemic occurred, and in a coffee man, a young man who came from Louisiana and who had periodic attacks all the time that he was up here, getting enough treatment for temporary abatement of the diarrheal symptoms. This patient shifted from one hotel to another, and thus kept a few months in advance of us, as we were examining the help at the various hotels, until we finally found him in the last hotel in which examinations were made.

TABLE 1—*Protozoa Found in the One Thousand One Hundred and Forty-Eight Food Handlers*

Types of Protozoa	Number of Cases 27 Carriers (2 active cases)	Percentage 2 35
<i>Endameba histolytica</i>		
<i>Endameba coli</i>	220	19 16
<i>Endolimax nana</i>	23	2
<i>Iodameba butschlii</i>	61	5 31
<i>Trichomonas intestinalis</i>	21	1 82
<i>Chilomastix mesnili</i>	22	1 91
<i>Giardia intestinalis</i>	75	6 53

He was immediately sent to the hospital, where he was kept until completely cured, as shown by proctoscopic examinations and repeated examinations of specimens of feces. The carriers were one vegetable salad girl, five fruit pantry girls, four cooks, three cold meat handlers, two bakers, one assistant fry cook, one waffle maker, one colored steward's assistant, one buffet man, three dishwashers, one toast maker and three pantry men.

Immediately on the discovery of a carrier or case, the steward was notified and the person was sent to the hospital and kept there until proved to be free from the organisms, which was done as follows. A series of treatments was given, followed by a three day period of rest without treatment, after which three negative stools on successive days were required before the patient was dismissed from the hospital. Failure to obtain three negative stools was followed by another series of treatments, a period of rest without treatment and three negative stools on successive days.

INCIDENCE OF PARASITIC INFECTIONS AMONG THREE HUNDRED AND EIGHTY NORMAL PERSONS

Similarly 380 persons were examined at the rate of 1 22 examinations per capita, the concentration method being used in addition to the method already described with the aim of uncovering as many cases of infection with eggs and worms as possible. These specimens were obtained largely from patients at the University of Illinois Research Hospital Dispensary.

The examinations gave the results listed in table 2

TABLE 2—*Protozoan and Metazoan Infections in Three Hundred and Eighty Normal Persons*

Types of Parasites	Number of Cases	Percentage
<i>Endameba histolytica</i>	4 (2 active cases)	1.05
<i>Endameba coli</i>	31	8.10
<i>Endolimax nana</i>	3	0.78
<i>Iodameba butschlii</i>	7	1.66
<i>Trichomonas intestinalis</i>	4	1.05
<i>Giardia intestinalis</i>	7	1.66
<i>Chilomastix mesnili</i>	6	1.57
<i>Trichuris trichiura</i>	4	1.05
<i>Oxyuris vermicularis</i>	2	0.52
<i>Trinia saginata</i>	2	0.52

COMMENT

The results of various workers in protozoology as well as our own work indicate that amebic dysentery is not limited to the tropics and subtropics. The present increase of amebic dysentery may be due to a number of factors, including (1) the greater stress being placed on medical zoology in the college curriculum, (2) improved clinical laboratory examinations, (3) increased migration from the Southern to the Northern states, and (4) the reporting of these cases as a stimulus toward more thorough examinations of diarrheal stools.

Stools of persons with amebic dysentery can often be recognized macroscopically as well as microscopically. Amebic dysentery stools are bloody mucoid free from macrophages and pus unless the case is of long standing, when secondary infections set in. On the other hand, stools showing a great deal of pus, rich in cellular exudate, some mucus and only now and then streaks of blood should be looked on with suspicion as being bacillary in nature. In all such cases bacteriologic examinations for dysentery bacilli should be made without any delay. Failure to find dysentery bacilli in cases of long standing does not rule out bacillary dysentery.

Extreme care should be exercised in examining freshly obtained specimens (preferably with a rectal tube or protoscopically) for amebas. This should be done immediately on discovering the symptoms and before treatment of the patient is begun. After specific treatment the organisms begin to disintegrate making it difficult or even impossible to determine them. Occasionally under such conditions stained preparations are of assistance. However all convalescents from amebic dysentery should be examined repeatedly for encysted *Endameba histolytica*. Periodic examinations should be made of the stools after the patients are discharged from the hospital.

EPIDEMIOLOGY FOR CHICAGO, 1926-1927, INCLUSIVE

By J. C. GEIGER

This investigation represents the first attempt to study epidemiologically cases of amebic dysentery reported to the Chicago Department of Health. The data are not complete even for the years under discussion since, as shown in table 3, some cases probably have never been fully reported. Likewise, in the assembled data there are noticeable gaps in the accumulated evidence due in part to the time that has elapsed since many of the cases were investigated. It is reasonable to assume, however, that this study did stimulate reporting by physicians and as a result there is presented an apparently marked increased endemicity for Chicago. Naturally, this

increase is of more than passing local interest, as it fully demonstrates the presence of the disease to a significant extent.

It must be appreciated, however, that this increase may be entirely due to recent careful investigation rather than to an unusual local parasitic index. Moreover, the extent to which the presence of amebic dysentery has been recognized in Chicago fully justifies this study and the plea to local practitioners to regard with suspicion all cases of chronic diarrhea and to confirm the clinical diagnosis of dysentery by suitable laboratory examinations.

Furthermore, once the local endemic index for amebic dysentery has been established by the reporting and laboratory confirmation of cases over a known period of years, the health official may determine how valuable the follow-up work, so necessary in such so-called imported diseases, has been in the elimination of possible foci of infection.

TABLE 3—*Dysentery in Chicago*

Year	AMEBIC	Cases	Deaths
1921			4
1922			2
1923			1
1924			6
1925			4
1926		63	11
1927		28	3†
	BACILLARY		
1921			1
1922			2
1923			1
1924			1
1925			2
1926		4	0
1927		3	0†
	UNSPECIFIED		
1921			2
1922			0
1923			0
1924			0
1925			0
1926		0	2
1927		0	0†
	TOTAL		
1921			7
1922			4
1923			4
1924			7
1925			6
1926		67	13
1927		31	3†

Ten months data
† Nine months data

SUMMARY

The number of cases in this study (150) is doubtfully exact. Particularly is this to be considered true because of the comparative importance of table 3. It will be noted that in the years 1921, 1922, 1923, 1924 and 1925 though deaths from both amebic and bacillary dysentery are recorded no cases are reported. The logical reason for this phenomenon is that probably physicians never regarded either disease as reportable except in 1926 and 1927, when this investigation awakened anew their interest at least in amebic dysentery, from a public health standpoint.

It is exceedingly important to note that practically the same number of deaths from amebic dysentery were reported for each year from 1921 to 1927, or a yearly average of 3.6 each, the exception being in 1926, when eleven deaths occurred. In the 150 cases recorded here there were eighteen deaths, or a percentage case mortality of twelve. In addition, twenty-nine carriers (two active cases) were discovered in the course of our investigation of food handlers in large local hotels.

Likewise, in this series (150) of cases, 106 were men, eighty-nine were women and five were children under

15 years of age. Eighty-three cases were verified by laboratory data, or a percentage of 55. This percentage of positive observations (55), however, has been mainly due to efforts of the past year to have corroborative laboratory observations in every case reported. It was interesting to note that of some of the epidemiologic factors brought to light, foreign travel figured in only fifteen patients, and two of these were born in foreign countries. An additional five were born in states of the United States bordering on the Gulf of Mexico. In a family of three, one of whom was a child, the possible etiologic factor was stated to be foreign missionary work. In the carriers discovered in the local hotel survey, however, eighteen, or approximately 64 per cent, were of foreign birth. In the remainder (ten) it is most interesting to note that these gave their place of birth as the United States and, in the majority, Chicago.

When occupation was considered as a possible epidemiologic factor, the investigation revealed a wide range, among which salesmen, housewives and laborers head the list. There were also included three physicians, one dentist and three nurses. Such occupations as broker, manufacturer, policeman, alderman, showman, musician, opera singer, machinist, houseman and painter were among those listed. Curiously, two restaurant employees were also listed. The most important epidemiologic factor, of course, as far as the Chicago cases are concerned, appears to be the carriers in local hotels. Our data as to this point are not susceptible of statistical analysis, as only five of the patients gave their residence as hotels in which carriers who were food handlers were discovered. Naturally, one would suspect that the carrier played more of a role than this in the epidemiology of amebic dysentery in Chicago, but the actual amount of harm done by these carriers will probably always remain obscure for the years under discussion.

CONCLUSIONS

1. Amebic dysentery is not uncommon in Chicago and has been frequently overlooked.

2. Careful examinations of freshly obtained specimens of feces in cases of colitis and diarrhea showing bloody mucus enable one to make the diagnosis easily.

3. In postdiarrheal stages diagnosis may usually be readily made by finding encysted *Endameba histolytica*.

4. Our results show that in Chicago the number of protozoan infections among food handlers is greater than among other persons taken at random.

25 East Washington Street

Child Welfare—In the last decade, community health programs have greatly expanded in their service to child welfare. This has come about mainly as a sequence to the development of preventive medicine. We are coming to realize more and more that, if constructive health is to be maintained, preventive measures must be instituted at an early age. The large number of school beginners with physical handicaps is striking evidence that such measures should have been carried out with these children in preschool life. Even though health workers are striving to attain the goal where children will meet standard health requirements before they enter school, this accomplishment is yet far from being attained. It will probably be for some time to come that large numbers of children will continue to begin their school career possessed of handicaps to their normal progress. It is of paramount importance, then, not to further delay finding these handicaps and in applying the necessary corrective procedures.—Allen, F. P. *Fixing the Responsibility for Corrective Work with School Children, Kentucky M J* December 1928.

PRENATAL PREVENTION OF POTENTIAL HEMORRHAGIC DISEASE OF THE NEW-BORN

I. NEWTON KUGELMASS, PH.D., M.D.

AND

JOHN E. TRITSCH, M.D.

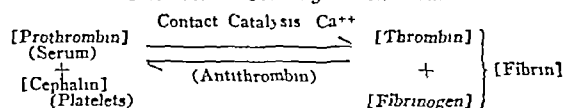
Associate Attending Pediatrician and Associate Attending Obstetrician
Respectively the Fifth Avenue Hospital

NEW YORK

True melena neonatorum is a distinct entity when hemorrhage from the gastro-intestinal tract and a progressively prolonged clotting time of the blood dominate the entire disease picture in a new-born infant. This disease differs from other hemorrhagic "diatheses" of the new-born secondary to sepsis, thrombocytopenic purpura, congenital syphilis, congenital anomalies of the viscera, and tumors of the abdomen.

The blood clotting time of true melena is not conclusive for characterizing the hemorrhagic tendency of a new-born infant. At birth the clotting time may be normal though spontaneous bleedings occur, and the clotting time may be delayed though there is no hemorrhage. The quantitative determination of the clotting components of the blood indicates the nature of its clotting deficiency and hence the hemorrhagic status of the infant. The blood deficiency of melena neonatorum is characterized by a marked decrease in the prothrombin content of the infant's blood. The methods for the quantitative determination of the substances involved in coagulation are reported elsewhere.¹

The Blood Clotting Mechanism



The hereditary aspect of abnormal bleeding has been restricted to hemophilia. Other forms of hemorrhagic disorders may be conveyed from parent to offspring without the transmission being sex-linked, but no studies of such disturbances have been made.

The blood of the fetus is in equilibrium with the maternal blood. Any deviation in the concentration of the clotting components would be manifest in the maternal blood studied early in the course of the pregnancy. The evaluation of the blood clotting function of maternal blood thus offers a basis of prediction of potential hemorrhagic disease developing in utero. Such an early diagnosis is more favorable for prenatal preventive therapy.

REPORT OF CASE

Mrs. T. N. had five pregnancies, the first four of which terminated in death from hemorrhagic disease in three new-born infants and possibly also in the fourth. The first baby, a girl, died about eight hours after birth; the second, a boy, was stillborn, following forceps delivery; the third, a girl, died within three hours after birth; the fourth, a girl, died at 22 days of age; the fifth, a boy, is living and normal.

The postmortem examination of the third baby showed icteric skin and sclerae and fresh blood over the mucous membranes. The middle fossa of the skull, brain, thymus, lungs, gastro-intestinal tract, bladder and rectum were hemorrhagic and bile stained. The spleen and liver were markedly hypertrophied. A diagnosis of hemorrhagic disease of the new-born was made. The fourth baby, spontaneously delivered at full term, weighed 8½ pounds (3.6 Kg.). At birth the skin was pale and jaun-

¹ I. Kugelmass, I. N. Am. J. M. Sc. to be published. Bancroft W. D., Stanley Brown, Margaret and Kugelmass, I. N. The Evaluation of the Blood Clotting Factors in Surgical Diseases. Arch. Surg. to be published.

diced the spleen was enlarged, and within six hours the temperature was 102 F. Whole blood was given intramuscularly followed by hyperdermoclyses. The white blood cells numbered 24,000, polymorphonuclears, 58 per cent, and platelets, 580,000. The red blood cell fragility began at 0.3 to 0.42. Blood began to ooze from the cord and vagina. Blood was present in the vomitus and stool. Successive transfusions from foreign donors, calcium salts, thromboplastin and other thromboplastic substances were without avail. Postmortem examination was denied.

The mother and father reacted negatively for syphilis and focal infections. During the fourth pregnancy the mother was toxic and was maintained on a low protein diet. The family history from the standpoint of hemorrhagic disturbances was negative.

The blood of Mrs. F. N. examined the second month of pregnancy showed a somewhat prolonged clotting time by tissue and by venous puncture. The prothrombin index was markedly low 0.31 (normal 1.0) as is characteristic in true melena neonatorum. The fibrinogen content was low 0.38 per cent (normal 0.6 per cent). The antithrombin index was somewhat elevated 1.5 (normal 1.0). The platelets were low normal 154,000. Hence Mrs. T. N.'s blood at the second month of pregnancy was poor in clotting function and specifically low in prothrombin content the characterizing deficiency factor in the blood of true melena. The father's blood was normal.

The clotting components of blood may be altered by nutritional measures. Prothrombin and fibrinogen are protein substances in libile dispersion in the plasma. Their concentration may be increased by high protein ingestion. The platelets are lipoidal protoplasmic separations from megakaryocytes. The platelet content of blood may be increased by a relatively high intake of varied lipins, abundant quantities of fat-soluble A, and small stimulative exposures to ultraviolet irradiation.

Clotting Factors of the Blood of the V Family

Date	Mother's Blood						
	Clotting Time	Bleeding Time	Prothrombin	Fibrinogen	Antithrombin	Platelets	Per Cent Lysis
December 1927			0.31	0.38	1.5	154,000	
March 1928	6'15" 1'15"	4'45"	0.25	0.75	1.4	175,000	37
May 1928	6'31" 1'45"	1'30"	1.12	0.79	0.7	175,000	6
Father's Blood							
May 1928	2'30" 1'31"	2"	0.18	0.46	1.0	25,000	70
Baby's Blood							
August 1928	17.5" 1'20"	1'20"	1.0	0.47	1.0	250,000	60

The treatment of Mrs. T. N. consisted in an analysis of her nutritional habits in order that her corrected food intake might be balanced and adequate and particularly high in varied proteins (6 Gm. per kilogram of body weight) and in varied lipins. The visceral organs were given because of their protein phospholipin content in this order lung, kidney, testes brain heart pancreas liver. The acid-forming proteins were neutralized by base-forming fruits and vegetables. Excess of basic salts is requisite for a positive calcium balance as well as for their favorable effect on prenatal development. On this regimen, the patient's blood became normal blood and a normal infant was delivered at term.

The nutritional regimen of Mrs. T. N. was as follows:

8 a. m., a thromboplastic substance, fruits and fructose and cream, coarse cereal and butter, eggs and bacon and acidophilus milk.

12 m., calcium chloride in spleen marrow 15 cc. vegetables, cooked vegetables, raw visceral organs bone marrow, raw, and fruit, stewed or raw.

6 p. m., calcium chloride in spleen marrow 15 cc. fruit and fructose and cream vegetable, cooked, acidophilus milk, and fruit, stewed or raw.

SUMMARY

1. A biochemical study of the clotting components of the blood from the second month of pregnancy to term in a woman who had given birth to five infants, three of whom, and possibly a fourth, had had true melena neonatorum, showed prothrombin deficiency analogous to that observed in true melena neonatorum.

2. Nutritional therapy of the mother throughout pregnancy developed and maintained a normal maternal blood before birth.

3. The fifth pregnancy terminated in the birth of a normal nonbleeding infant.

4. This case is suggestive of the possible value of prenatal treatment in bringing about the physiologic perfection of the new-born.

Further work on this subject is in progress.

15th Avenue and One Hundred and Fifth Street

COMPARISON OF LEUKOPLAKIA MALAKOPLAKIA, AND INCRUSTED CYSTITIS

REPORT OF CASES AND A NEW METHOD OF TREATMENT*

FRANCIS H. REDEWILL, M.D.

SAN FRANCISCO

Incrusted cystitis, leukoplakia and malakoplakia, and the rarest and most interesting of these is malakoplakia. All three are typical urinary bladder infestations, and while the latter two may occur in other parts of the urinary tract as the kidney pelvis, ureters or urethra the second pathologic condition mentioned, leukoplakia, is not uncommonly seen in the buccal and nasal cavities and also involving the conjunctiva. In Liebenow's¹ case there was involvement of the diaphragm.

Although the resultant pathologic lesions of these bladder conditions do not resemble one another the inherent basic process underlying the formation of the lesions is strikingly similar in all three types, and for that reason I will briefly compare the three as to pathology also giving a demonstration of the actual lesions, and finally will present a new interesting method of treatment.

All the lesions are concomitant with chronic inflammatory processes of the urinary bladder of the mucous membrane, or interstitial tissue, or both. Most interesting is the fact that calculeous deposits play an important part in the pathologic processes of all three malakoplakia, leukoplakia and incrustated cystitis. For instance in malakoplakia in the large myelotic cells of the mucous membrane are found the Michaelis-Gutmann bodies² that look like corpora amylacea and are calcium phosphate inclusions. In the epidermization of leukoplakia, calcium plays an important part not only in the formation of the stratum corneum but also in every process of the formation of the thickened peril gray, glistening membrane bits of which, when passed by the patient are spoken of as "gravel." Lastly, calcium salts play a paramount role in the formation of incrustated cystitis the incrustations being almost pure calcium salts. This rather common malady is manifested by one of two types of deposits, namely, thin

* Read before the San Francisco County Medical Society Urological Section May 22, 1928.

1 Liebenow, A. Beitr. z. klin. Chir. Festschrift für Czerny 1907.
2 Michaelis and Gutmann. Ueber Einschlüsse in Blasenentzündungen. Ztschr. f. klin. Med. 47: 208, 1902.

calcified plaques, characteristic of formation in acid urine, and thick, vacuolated, white tumor calcium masses found more commonly in alkaline urine.³

MALAKOPLAKIA

Malakoplakia was named by von Hansemann⁴ in a carefully written article in 1906, but was first described by Michaelis and Gutmann in 1903. Since then there have been several excellent German articles describing this rare condition of the urinary bladder, of which the most recent, with a careful pathologic description, is by Oppermann.⁵ An excellent description of this lesion with colored plates is that by McDonald and Sewell,⁶ published in Great Britain. The first American writing on the subject was by Pappenheimer.⁷

Malakoplakia means soft plaques, and the condition is characterized by brownish raised areas of the mucous membrane of the bladder, it also occurs in the kidney pelvis and the ureters, and the size of the plaques varies from pinhead single nodules to large coalesced masses with a spread of from 3 to 4 cm and raised from 1 to 2 cm above the normal mucous membrane. These growths are soft and have a tendency to center umbilication because of the breaking down of stratum germinativum and granulosum in the mucous membrane layers, with resultant ulceration. Oppermann⁵ recently reported forty cases of malakoplakia, twenty-eight in women and twelve in men, almost all of them being past 40 years of age. There have been three cases reported in children, including Oppermann's case in a girl of 8.

The epithelium of the mucous membrane at the center of the prominences is entirely lacking. On the slopes it is pushed a little upward, giving the impression that the raised area developed under the mucous membrane and, in growing, eroded it. Almost entirely occupying the tumor structures are the macrophage-like large cells in which and around which are seen refractive rounded bodies containing calcium and iron salts. These bodies were first described by Michaelis and Gutmann. These authors² considered the multiple raised areas as true tumors, but Guterbock,⁸ Hart,⁹ Landsteiner and Stoerk¹⁰ and Gierke¹¹ showed that it was a definite

inflammatory process of the bladder wall. A new point of view was brought into the whole discussion when Kimla¹² showed that tubercular changes of the vesical membrane can offer microscopically, completely or partly, a picture which answers that of malakoplakia. The case described by Wildbolz,¹³ which was tuberculous cystitis, was erroneously called malakoplakia. Zangemeister¹⁴ went still further in his erroneous deductions in stating that a case could be called malakoplakia only if, on the finding of rounded caseous cystic plaques in the bladder, the patient showed a positive tuberculin reaction.

It is true that in the list of cases compiled by Oppermann,⁵ in twenty-eight out of forty there had been made a diagnosis of pulmonary tuberculosis. The frequency of tuberculous changes of the lungs during advanced age has to be taken into account here. In twelve of the cases no tuberculous involvement could

be found post mortem. Another question is whether or not the bacteria found by a majority of the observers play the rôle of specific stimulators in the genesis of the disease. Landsteiner and Stoerk, as well as Hart, observed that in the stratum germinativum, or deepest layer of the mucous membrane, there were numerous large cells filled, as it were, to the brim with bacteria. However, in the cases of Guterbock,⁸ Loth,¹⁵ and Oppermann no bacteria whatever could be found in the tissues. The two distinctive cardinal observations that are necessary for making a diagnosis of malakoplakia, as has been pointed out, are the numerous large macrophage cells and the rounded incrustations or corpora amalycea-like bodies,

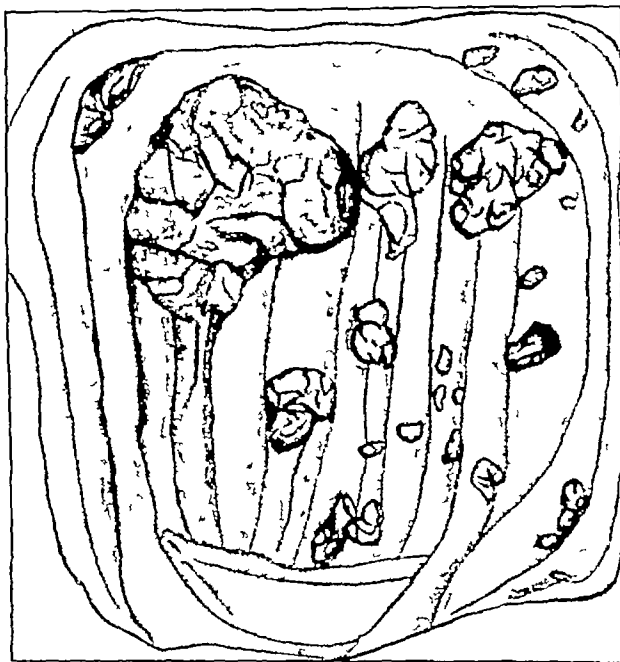


Fig 1 (case 1)—Malakoplakia of the urinary bladder (From a painting by Hamilton)

many intracellular and some extracellular. If bacteria are found in the tissue one must consider the possibility that there existed simultaneously an acute or a chronic cystitis. But one must remember at this point that advanced stages of malakoplakia may exist in the urinary bladder and yet there may not be any symptom of cystitis, as in the cases of Loth,¹⁵ Guterbock⁸ and Oppermann and in my own.

There is considerable discussion in the literature as to the origin of these characteristic large malakoplakia cells. Most of the authors previously thought that they were from the spindle cells of the connective tissue, von Hansemann and Hart⁴ considered that they originated in the endothelium of the lymphatics, Gierke,¹¹ that they were primarily interstitial cells swelled with cystic

¹² Kimla. Hansemann's Malakoplakia vesicae urinariae und ihre Beziehung zur plaqueförmigen Tuberculose der Harnblase. Virchows Arch. f. path. Anat. 184: 469 1906.

¹³ Wildbolz. Plaqueförmige Tuberkulose Cystitis unter dem Bilde der Malakoplakie vesicae. Ztschr. f. Urol. 1: 322 1907.

¹⁴ Zangemeister. Ueber Malakoplakie der Harnblase. Ztschr. f. Urol. 1: 877, 1907.

¹⁵ Loth. Ueber zwei besonders seltene Fälle von Harnblasen Affektion. Monatsch. f. Urol. 11: 90 1906.

³ Cabot. Textbook of Urology.

⁴ Von Hansemann and Hart. Ueber Malakoplakia der Harnblase. Virchows Arch. f. path. Anat. 173: 302 1903.

⁵ Oppermann, Ernst. Malakoplakia of the Urinary Bladder in a Girl of Eight Years. Ztschr. f. Urol. 38: 365 1926.

⁶ McDonald, Stewart and Sewell W. T. Malakoplakia of the Bladder and Kidneys. J. Path. & Bact. 18: 306 1913 1914.

⁷ Pappenheimer. Malakoplakia of the Urinary Bladder. Report of Two Cases. Proc. New York Path. Soc. 6: 1906.

⁸ Guterbock. Ein Beitrag zur Malakoplakie der Harnblase, Diss. Leipzig 1905.

⁹ Hart. Ueber die Malakoplakia der Harnblase, Ztschr. f. Krebsforsch. 4: 380 1906.

¹⁰ Landsteiner and Stoerk. Ueber eine eigenartige Form chronischer Cystitis, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's) 36: 131 1904.

¹¹ Gierke. Ueber Malakoplakia der Harnblase. München med. Wechnsch. 29: 1388 1905.

urein, but Miche¹⁶ disproved this by showing that they were not degenerative cells but highly active and phagocytic. Krompecher,¹⁷ Miche¹⁶ and Oppermann⁵ hold to the more tenable view that they are macrophages in the Metchnikoff sense. As to the corpora amylicae-like deposits known as Michaelis and Gutmann bodies, it seems to be an established fact that they are composed of calcium salts absorbed from the urine and that their formation is directly stimulated by bacterial action. Loelke¹⁸ proved this in the following experiment: To normal human urine was added an equal quantity of bouillon, the mixture was infected with *B. coli* and fresh blood was added. After ten days there were found in the fluid great quantities of typical encystations or refractile lime granules.

As to the physiopathologic action that produces the resultant malakoplakia nodules. With a primary irritant, bacterial or otherwise, in the wall of the bladder or urine or both, there is stimulated the absorption of

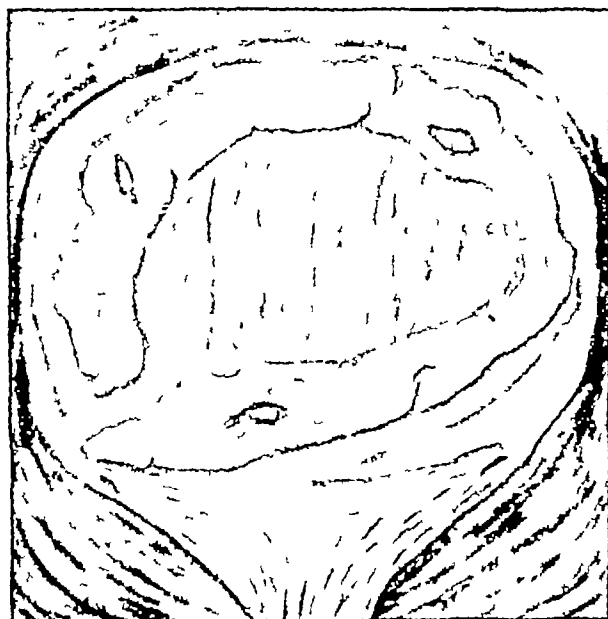


Fig. 2 (case 2)—Leukoplakia of the urinary bladder (From a painting by Hamilton)

lime salts from the urine within the mucous membrane which are deposited as the Michaelis-Gutmann bodies, and which, together with the primary irritant, induce the impouring of macrophages. Large numbers of these bodies and phagocytes, and also the typical basal lymphocytic infiltration, cause a bulging of the mucous membrane. At the peak of these nodules, because of insufficient blood supply, there is a tendency to umbilication and ulceration.

The following is a report of the only case of malakoplakia that I have seen.

CASE 1—A man, aged 62, with hematuria but without symptoms of cystitis, was examined in June 1927, and found to have chronic pulmonary tuberculosis in the primary stage in the right upper lobe, chronic arthritis and hypertrophied prostate with blood, there were albumin and casts in the urine but bacteria were not demonstrable after continued search; there

was no frequency, tenesmus, or residual urine, and pyelograms taken on each side showed the pelvis to be negative as to pathologic signs. Four lumps of the kidney pelvis with weak silver nitrate solution cleared up the hematuria.

There were numerous raised, rounded, brownish soft plaques varying in size from that of a pea to one coalesced nodule in the upper right portion of the bladder wall with about 3 cm spread (fig. 1). The plaques did not show ulceration. Removal of a small plaque with cystoscopic rongeur frozen section showed typical malakoplakia cells and Michaelis-Gutmann bodies. A diagnosis of malakoplakia of the urinary bladder was made. The patient was treated for four months. Some of the nodules disappeared and the remainder decreased in size. The treatment used will be described here later. The patient went to New Mexico for the pulmonary condition.

LEUKOPLAKIA

The word leukoplakia means white plaques or white plates and, as has been pointed out, it is essentially an epidermization, a development of mucous membrane on mucous membrane. It is a rare disease of the bladder and kidney as compared to leukoplakia of the membranes of the cheeks, of the gums and of the tongue.

Briggs and Maxwell¹⁹ believe that the present greater cooperation between pathologist and surgeon and the greater number of necropsies in America now as compared to the past will lead to a rapid increase in the number of cases reported. These authors, in a recent paper, review eighty cases thus far reported, the condition in forty-three being vesical and in forty renal, but they believe that the disease occurs more often in the

Associated Pathologic Conditions in Leukoplakia

Tuberculosis	7	Stone in opposite kidney and ureter	1
Hydronephrosis	24	Stone in bladder	3
Chronic pyelonephrosis	25	Foreign body in kidney	1
Stone in kidney	10	Carcinoma of kidney pelvis	1
Stone in ureter	2	Carcinoma of bladder	3

kidneys than in the bladder because the diagnosis of most kidney disease depends on operation or necropsy, whereas the diagnosis of the bladder condition can be made by cystoscopic examination. Unlike malakoplakia, leukoplakia occurs twice as often in males as in females, however, both types of lesions in the great majority of cases have been reported in patients over 40 years of age. Three exhaustive American articles on the subject have been written by Kretschmer²⁰ and by Hinman and his co-workers.²¹ The first article on the subject was published by Rokitsky in 1861 and the first report in the American literature was by A. T. Cabot in 1891, thirty years later. The accompanying table shows the pathologic conditions associated with leukoplakia.

THEORIES OF LEUKOPLAKIA

The theories of leukoplakia formation have been thoroughly reviewed by the authors mentioned. Briefly stated, the two outstanding theories are those of Lecene, who believed that the condition was of congenital origin and the result of misplaced cell rests, and of Haythorn, who thought it was due to chronic irritation. The latter author concludes that "the mucosa, destroyed beyond the hope of specific regeneration, seeks to protect itself with the best reparative cells it can produce in its

19 Briggs W. T. and Maxwell C. S. Leukoplakia of the Urinary Tract. Report of One Vesical and Two Renal Cases. *J. Urol.* 18:1 (July) 1926.

20 Kretschmer H. L. Leukoplakia of the Bladder and Ureter. *Surg. Gynec. Obst.* 31: 325 (Oct.) 1920. Leukoplakia of the Kidney Pelvis. *Arch. Surg.* 51: 348 (Sept.) 1922.

21 Kutzman A. A. Gibson T. E. and Hinman Frank. Leukoplakia of the Kidney Pelvis. *Surg. Gynec. Obst.* 30: 472 (Oct.) 1924.

16 Miche cited by Gierke (footnote 11).

17 Krompecher. Ueber Malakoplakie der Harnblase. *München med. Wehnschr.* 28: 952, 1904.

18 Loelke. Ein Beitrag zur sog. Malakoplakie der Harnblase. *Beitr. z. Path. Anat. u. z. allg. Path. (Ziegler's)* 48: 205, 1910.

exhausted state, furthermore, that there is probably a link between the injury to the basement membrane and tumor growth." Richie, Briggs and Maxwell express the same idea in different words. There may be an inherent but latent ability to undergo metaplasia, acquired in the processes of evolution in the epithelial cells when the environment is such that protection is necessary.

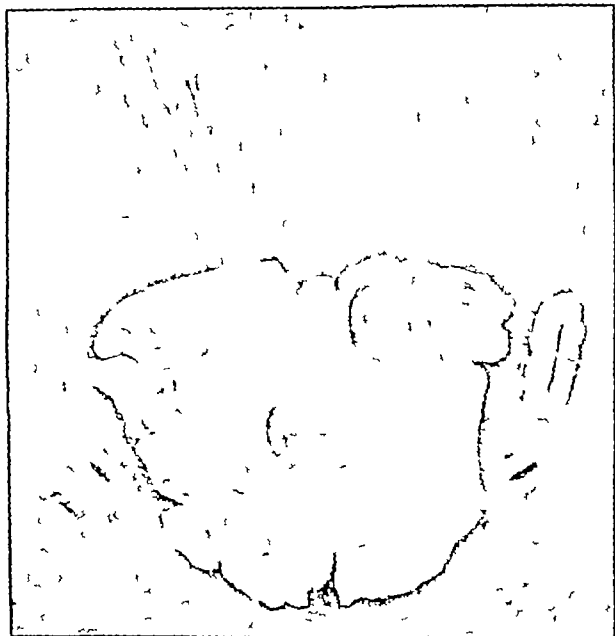


Fig 3 (case 3)—Leukoplakia of the urinary bladder (From a painting by Hamilton)

The first of the following brief reports of two cases of leukoplakia is Dr Player's, the second, mine.

CASE 2—Mrs L, aged 35, had had a rebellious cystitis for a year prior to examination, and had passed "gravel." There were no complications. Cystoscopy showed an injected bladder mucosa with a pearly gray, glistening, narrow, continuous plaque extending from the right ureteral orifice in a circular manner well up toward the fundus and around toward the same orifice (fig 2).

CASE 3—Mrs S., aged 63, appeared robust but had had an aggravated cystitis for years. Cystoscopy showed a large square leukoplakia growth in the bladder lying on the trigon between the ureteral orifices and extending toward the fundus (fig 3). The treatments in these cases will be discussed later.

INCRUSTED CYSTITIS

Caulk made the first report on incrustated cystitis in 1914 before the American Association of Genito-Urinary Surgeons. The condition is essentially an alkaline cystitis characterized by two types, the flat or surface type, and the elevated or tumor-like type. These may occur alone or may be associated.²² The elevated tumor-like mass is the rarer and is due to the constant precipitation of salts on the already incrustated ulcer. The incrustations are very adherent to the bladder wall and on removal leave a punched out ulcer with irregular borders. The incrustations are usually limited by the submucosa, but the whole bladder may be involved in advanced cases. The thin, shell-like formations occur in acid urine, whereas the thick, tumor-like calcifications occur in alkaline excretions. Rapid occurrence even within forty-eight hours after removal is the pathologic characteristic of this disease. Pyelonephritis is a common complication.

CASE 4 (Dr Player's case)—A man, aged 65, had incrustated plaques on the dorsal portion of the bladder above the trigon (fig 4). He was treated every two weeks for twenty-six months with fulguration, topical application, and bulgarian bacillus emulsion, and is still continuing treatment.

CASE 5—A woman, aged 40, who appeared robust, had had a very acute cystitis for one year. Cystoscopy showed thin incrustated plaques spread over the base of the bladder involving the right ureteral orifice. The patient developed pyonephrosis in the right kidney, and a nephrectomy was done. The regular routine treatment of removing plaques with rongeur, fulguration, topical application, and the use of bulgarian bacillus emulsion did not check the condition. The new treatment mentioned here cleared up the condition in a comparatively short time.

TREATMENT

These three types of urinary bladder infestation are grouped together in this paper not only because they resemble one another in the one particular of lithiasis formation and the epidermization of leukoplakia, but also because it was found that a certain line of treatment helped in all three types. Osborne and Mendel²² reported that rats on a diet deficient in vitamin A had a tendency to develop calculi in the urinary tract. They wrote, "In every instance where calculi developed, the animals were without an adequate source of fat soluble vitamin for some time." This work was confirmed by Van Leersum of Amsterdam.²³ Another interesting truth regarding the etiology and treatment of leukoplakia is the tendency to keratinization of mucous membrane epithelial cells with the absence of fat-soluble vitamin from the food.²⁴ With these facts in mind, it occurred to me to treat the patient with leukoplakia with



Fig 4 (case 4)—Incrusted cystitis of the urinary bladder (From a painting by Hamilton)

forced feedings of vitamin A. This was continued for about six months but the expected rapid improvement did not occur.

22 Osborne T B and Mendel L B The Incidence of Phosphatic Urinary Calculi in Rats Fed on Experimental Rations J A M A 69: 32 (July 7) 1917

23 Van Leersum, E. C Vitamin A Deficiency and Urolithiasis J Biol Chem 76: 137 (Jan) 1928

24 Urolithiasis and Deficiency in Vitamin A, editorial J A M A 90: 1297 (April 21) 1928

SYMBIOSIS

No report has yet been published on the subject of symbiosis of vitamins, but a noted dietitian²⁵ stated recently that a condition of symbiosis exists. In the feeding of vitamins, an interrelation is present to such an extent that each vitamin aids the others in their potency of action on the patient and in many cases the pushing of one vitamin will not produce any results unless careful feeding of all the vitamins is also observed. In view of this valuable advice I urged my patient to be careful to use all the vitamins in her food and continued to push vitamin A. Cameron²⁶ has also shown how injections of parathyroid will correct the abnormal drainage and deposition of calcium salts in patients with the tendency to epidermization and the formation of incrustations. Other reports that seemed to make it all the more logical to use parathyroid extract in these bladder infestations were those dealing with (1) the curing of duodenal, buccal and bladder ulcers by means of parathyroid treatment, (2) the tendency for cataracts to form in patients with hypoparathyroidism, and (3) the actual curing of several patients with leukoplakia of the tongue by the administration of parathyroid.²⁷

Whereas the patient with leukoplakia was treated for fourteen months without much improvement even while the vitamin A diet was being pushed, on a careful combination of the other vitamins B, C, D and E to the fat-soluble A food along with routine treatment, the condition cleared up in six weeks. The plaques, however, had a tendency to recur if treatment was stopped and especially if the patient became lax in her diet. This tendency to recur was checked by giving 20 units of parathyroid three times a week. The combined vitamin diet and parathyroid therapy rapidly reduced the size of the malakoplakia nodules. Both were given with routine treatment. Also the lesions cleared up rapidly under this combined treatment, together with the routine treatment of removing the plaques by fulguration and cauterizing with 20 per cent silver nitrate by the Player method.

In the case of incrustated cystitis there was a tendency to recurrence, and the patient suffered from acute cystitis. By pushing vitamin A, and also by giving the other vitamins and administering parathyroid, the tendency for the incrustations to recur was completely checked. The parathyroid was administered for about five weeks. It should be emphasized here that should there be also a coexisting renal infection that is incurable, or if a malignant condition exists—advanced leukoplakia, as is well known, being prone to become malignant—it is useless to hope to obtain a cure by the conservative treatment outlined here. It must be remembered that the chronicity and depth of the infestation has a great deal to do with the rapidity of the cure. Since malakoplakia involves the entire mucosa and even the submucosa, diathermy is a useful therapeutic adjunct. When certain infective bacteria is causative factors in these various infestations can be isolated, the new ecto-antigens help materially to clear up the infections.

COMMENT

In reporting a new line of treatment it is scientifically more nearly accurate to report the use of such a treatment in a large series of cases. However because of the rarity of leukoplakia, and malakoplakia especially,

probably no such large series, treated by one investigator, will ever be obtainable. I can here report my success in the treating of only one of each type of the bladder infestations. It remains for further animal experimentation and the future reports of many urologists to corroborate the marked success obtained in these types of bladder cases with the use of parathyroid and the vitamins.

SUMMARY

1 Of the two cases of incrustated cystitis, two cases of leukoplakia of the bladder and one case of vesical malakoplakia here reported, two cases, one of incrustated cystitis and one of leukoplakia, were Dr. Player's.

2 To warrant a diagnosis of malakoplakia, a portion of the plaques removed should show macrophage cells and Michaleis-Gutmann bodies.

3 Cases of malakoplakia are rare, only forty-four cases having been reported in the literature to date, including nine. Eighty-eight cases of leukoplakia have been reported, whereas incrustated cystitis is of rather common occurrence.

4 These three infestations of the urinary bladder resemble one another in that calcium salts are deposited and there is a tendency to epidermization.

5 The pushing of all the vitamins in the diet, and the injecting of parathyroid aid materially in the treatment of all three of these conditions.

6 Valuable adjuncts in the treatment are the Player method of cauterizing, diathermy and ecto-antigen injections.

7 Malignant changes in the vesical plaques and blocking of the ureters are grave complications. However, if the lesions have not advanced to the malignant stage, even involvement of the kidneys and ureters—provided the ureters are patent—need not necessarily portend a bad prognosis, if parathyroid and vitamins are administered early with routine treatment.

8 When parathyroid is administered, accurate determinations of the serum calcium should be made to avoid overdosage, i. e., symptoms of hypercalcemia.

686 Flood Building

Facts Facing the Profession.—The air is full of legislative and other panaceas for the one outstanding social and economic fact, i. e., we have not devised a financial system by which all members of society, regardless of economic status, may receive a full or even a reasonable share of the benefits possible through the practical application of modern scientific medicine. Our distribution is faulty because our methods are behind the times and the public thinking is addled. What other business does free work as a part of its regular program? What other business has a sliding scale of charges varying from \$25 to \$10,000 for the same service? Who knows how much it costs a physician to make a call? We know what cost accounting has done for modern business. Who knows anything about cost accounting in medical practice? We have not even the evidence upon which to develop a modern plan and yet a considerable proportion of the profession are fundamentalists with locked minds when any new procedure is proposed. We cannot escape change. The question is, are we going to guide it?

How can we keep that independence which is essential in the life of the physician and also that close personal relationship between doctor and patient which is vital to successful care of a large proportion of human ailments? Some plan must be devised so that official snoopers will not be projected in between doctor and patient. The physician must show that acute and yet conservative responsiveness that is the basis of the rapid progress of medicine as a science in the development of the practice of medicine in an economic world with new concepts of government, social organization, publicity, education and science—Wilbur R. L. The Cost of Medical Care, *California & Western Med.*, July, 1928.

25 Bidding A. W. Personal communication to the author.

26 Cameron, A. T. *Biochemistry of Calcium*. Canad. M. A. J. 16: 753 (July) 1926.

27 Yoell R. A. Personal communication to the author.

AMEBIC ULCER OF THE ABDOMINAL
WALL FOLLOWING APPENDECTOMY
WITH DRAINAGE

REPORT OF CASE *

WARREN H. COLE, M.D.
AND
MILO L. HEIDEMAN, M.D.
ST. LOUIS

Several reports of progressively enlarging gangrenous ulcers of the abdominal wall following laparotomy (usually appendectomy with drainage) have been observed in the literature during the last few years, but in no instance has the etiologic factor been definitely demonstrated. In the case described in this report, the ulcer that followed an appendectomy with drainage was conclusively proved to be amebic in origin. Amebas were found in the pus that exuded from the ulcer of the abdominal wall and were demonstrated in a microscopic section taken from the actively growing edge of the excised ulcer. Although in this patient there had never been a positive history of diarrhea which might be classified as amebic dysentery, the origin of the amebic organisms seems surely to have been from the infected appendix. We cannot offer any proof that the amebic infection had anything to do with the onset or course of the appendicitis, but the possibility of its etiologic relationship remains. Numerous large poorly defined bodies were found within the wall of the appendix near the mucosa, but they could not be identified as amebas as definitely as the organisms in the pus and wall of the ulcer. The possibility of amebas being the cause of the ulcer was first suggested by Dr. Richard Weiss, who saw the patient in consultation. The organisms were found in the pus at that time by Drs. Weiss and Heideman. The patient was later seen by Dr. M. F. Engman, who agreed without reservation to the diagnosis of amebiasis cutis.

The diagnosis of amebic ulcer was made possible by a report of three cases of amebiasis cutis by Engman and Heithaus¹ in 1919. Their first patient was suffering from a severe diarrhea. The discovery of amebas in the stool led them to conduct a similar search, which was successful, in the pus draining from the numerous large ulcers scattered over the body. The ulcers present in their cases were very similar to the ulcer of the abdominal wall in our patient.

REPORT OF CASE

History—A white man, aged 34, was admitted to the Barnes Hospital, May 24, 1927, with the complaint of severe pain in the right part of the abdomen which had begun twelve hours before admission. The pain first appeared in the epigastrium but later confined itself to the right side of the abdomen opposite the umbilicus. The bowels moved two or three times shortly after the onset, but there was no previous history of diarrhea. The patient vomited several times from eight to ten hours after the onset of pain. The pain was unusually severe for appendicitis. There was nothing abnormal in the genitourinary history. There had been no jaundice at any time. There was nothing of significance in the past or family history except for a similar but milder attack of abdominal pain which had occurred a few years previously. No members of the family had ever had amebic dysentery. The patient had been occupied as a baker for the past ten or twelve years.

Physical Examination—The temperature was 37.4 C. (99.3 F.), the pulse, 76, and the respiration, 22. There was a moderate muscle spasm over the entire right side of the abdomen, with tenderness in the same area, localized chiefly to the right of the umbilicus. There were no masses or distention. Abnormalities were not found in the heart or lungs.

The white blood count was 11,000 but rose to 16,000 just before the operation. The red blood count was 4,480,000. The hemoglobin was 80 per cent. The urine was normal.

Operation—May 24, twenty hours after the onset of pain, operation was performed. The appendix was found under the cecum, fairly well walled off, but indurated, red and considerably enlarged. The appendix contained one large fecalith and had ruptured at this point. A communication between the lumen of the cecum and the lumen of the proximal portion of the appendix suggested a perforation into the adherent cecum.



Fig. 1—Appearance of ulcer ten weeks after onset. Note beginning invasion on the left side of the abdomen but freedom of the umbilicus and operative scar from any involvement. Two or three islands of epithelium can be seen scattered over the surface of the granulating base. Growth was upward in the direction of the lymph and blood flow and never downward.

during the previous attack. The appendix was removed and the stump inverted. The wound was closed around a rubber drain leading down to the appendical stump.

Course—The first few days of convalescence from the appendectomy were very smooth. There was very little drainage, and the temperature was normal.

May 29, manipulation of the drain caused so much pain that the administration of nitrous oxide anesthesia was necessary for its removal.

June 11, eighteen days after operation, a small, red, indurated, tender area appeared at the site of entrance of the stay suture, lateral to the scar of the wound. It resembled a large furuncle in every detail. The sinus at the drainage site had ceased draining several days previously and was now completely healed.

June 12, the patient was discharged.

June 20 there was a slough in the supposed furuncle which was separating slowly and enlarging. Culture revealed a hemo-

* From the Departments of Surgery and Internal Medicine, Washington University School of Medicine and the Barnes Hospital.
¹ Engman, M. F. and Heithaus, A. S. Amebiasis Cutis. J. Cutan. Dis. 37:715 (Nov.) 1919.

lytic staphylococcus on two or three occasions. There were a few contaminating rods.

July 20, the ulcer was slowly enlarging. Hot, wet packs were administered. Irrigation with surgical solution of chlorinated soda (Dakin's solution), mercurochrome packs, ultra violet ray therapy, infra red therapy and sunlight were tried.



Fig. 2—Artist's drawing of the amebas in the pus draining from the ulcer. The average diameter of the organism was from eight to ten times that of the ordinary leukocytes which are depicted alongside the amebas. Note the pseudopodia.

at different times after the onset of the ulcer, but improvement was not noted. There was extreme tenderness and pain about the ulcer.

July 22, the diagnosis of amebiasis cutis was suggested by Dr. Richard Weiss. Amebas were found in the pus draining from the ulcer, which now measured about 12 cm. by 18 cm. The patient was started on a course of emetine hydrochloride subcutaneously and acetarsone by mouth.

July 24, there was a very definite improvement in the appearance of the ulcer. Induration and redness were considerably decreased, which was attributed to the emetine.

September 1, after the administration of 35 Gm. of acetarsone over a period of about five weeks, the patient developed an arsenic rash. Acetarsone was discontinued. Much morphine was required daily for the relief of pain.

September 20, it was noted that the patient had received 24 grains (15 Gm.) of emetine hydrochloride subcutaneously during the previous eight weeks. During this time emetine was discontinued twice for seven or eight days on account of possible toxic symptoms resulting from its administration. Cessation of emetine therapy always resulted in a more active growth of the ulcerating process.

October 19, the patient was admitted to the Barnes Hospital for x-ray therapy, which was given by Dr. Sherwood Moore. Another course of emetine was begun at this time. Repeated examinations for amebas in the stool were negative.

Very definite improvement was noted on the fourth day following x-ray therapy and the second course of emetine, but active growth and extension began shortly afterward in the part of the ulcer on the left side of the abdomen.

In previous consultations with Dr. E. A. Graham and Dr. V. P. Blair, cautery excision had been chosen as the most logical method of treatment if emetine and x-ray therapy proved inadequate. Accordingly, November 7, cautery excision of the edges of the ulcer on the left side of the abdomen was done, a margin of about 1½ inches of skin being removed. The base of the ulcer adjacent to the skin was excised with the skin. A gauze dressing soaked with surgical solution of chlorinated soda was applied.

November 19, the patient was discharged from the hospital. Granulations and epithelium were growing very rapidly. There was no evidence of recurrence of the ulcerative process.

Appearance of the Ulcer—The lesion started as a red spot seventeen days after appendectomy at the site of exit of a stay suture and resembled a furuncle. The central portion became gangrenous, causing a slough, which separated slowly. The skin surrounding the slough became red, indurated and very tender. In a few days the margin assumed a pearly appearance with a cyanotic blush, and within a few more days a grayish slough was formed with an occasional area of dark discoloration. This slough included only the skin, and, after it separated, left a smooth, red, granulating surface. The ulcer grew in size by means of this sloughing process, and before it finally was subdued had covered practically the entire right side of the abdominal wall and the upper portion of the left side. The edges were very irregular, and extremely tender and painful. There was a distinct tendency for the ulcer to spread upward toward the head in the direction of the venous and lymphatic flow, with a disposition to heal by scarring and epithelization in its inferior portions. The granulating base was dotted here and there with small islands of epithelium, some of which were very thin and presented a translucent appearance. At no time did the ulcerating process break across the scar of the suture line or invade the umbilicus.

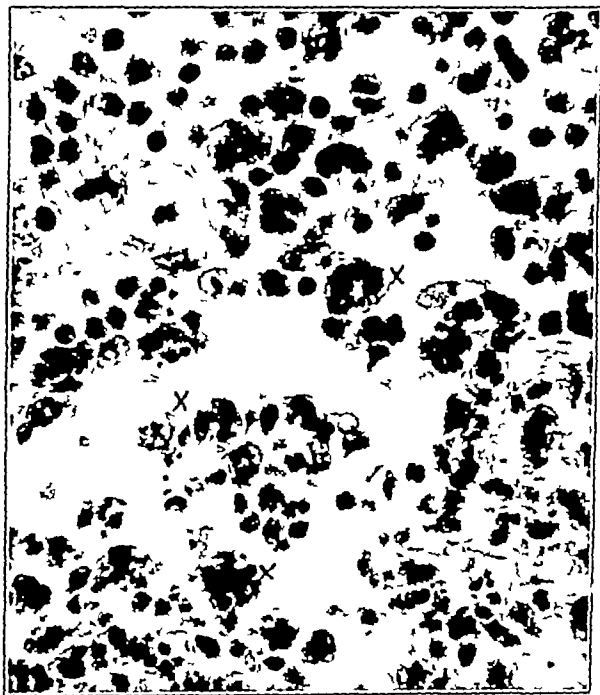


Fig. 3—A cluster of the amebas on the edge of a large microscopic abscess in the section obtained from the actively growing ulcer of the skin. X marks the location of the organisms in focus. Numerous others were present. Note that many of the organisms are engorged with leukocytes as well as erythrocytes.

Nature of Organism—Sluggishly motile amebas were found in large numbers in the pus draining from the ulcer. After administration of the first few doses of emetine the motility was practically entirely lost, but organisms could always be found. Most of them assumed an irregular shape with pseudo-

podia, even after they lost their motility. They were many times larger than a polymorphonuclear leukocyte, averaging about 25 microns in diameter. The protoplasm was granular, contained numerous vacuoles and, in many instances, had a refractory, almost glassy, appearance. The nuclei varied from 4 to 7 microns in diameter, and contained chromatin bodies as well as coarse granular material. Particles of red blood cells were occasionally seen in the protoplasm. A few of the organisms were round or oval, small and without pseudopodia, these probably represented the encysted form.

The organisms found in the stained sections, taken from the skin at the edge of the sloughing portion of the ulcer, presented different characteristics. Their relatively small size with round or oval shape probably indicated that practically all were of the encysted form. A characteristic of the organism isolated in this case, which is unusual for any kind of ameba described in the literature, was its ability to engulf lymphocytes, and even polymorphonuclear leukocytes, in large numbers. Frequently the amebas would be engorged with these blood cells and contain only a relatively small number of red blood corpuscles, most of which were fragmented. An ordinary hematoxylin and eosin stain yielded satisfactory staining of the amebas. Giemsa and polychrome methylene blue (methylthionine chloride, U. S. P.) stains were slightly less satisfactory. There was a very definite tendency for the organisms to occur in clumps, but these clumps occurred only in certain areas throughout the sections. Usually they were located about the periphery of large collections of pus cells. Only occasionally could they be found isolated among the tissue cells.

COMMENT

Search of the literature revealed at least five other cases which were so nearly identical in detail as to lead one to believe that the etiology of these particular cases must be the same. The first one was reported by Cullen² in 1924. It also occurred after an appendectomy with drainage, and was eradicated only after the edge of the actively growing ulcer was excised with the cautery. *Streptococcus brevis* was the only organism found by culture.

Shortly after this, Christopher³ reported a similar lesion which began as a red, indurated area similar to a furuncle, thirteen days after a rib resection for empyema. The edges of the ulcer that developed were also red, indurated and excruciatingly tender and painful. A direct smear of the pus from the pleural cavity revealed a gram-positive coccus and a gram-negative bacillus. The fact that *B. coli* was at one time isolated from the pus (on the forty-first postoperative day) might indicate that there was some indirect connection with the intestinal tract.

From the ulcer in a case reported by Brewer and Meleney,⁴ a streptococcus and a staphylococcus were isolated which, when injected just beneath the skin of animals, produced a gangrenous slough in a large percentage of the injections. Brewer and Meleney felt that perhaps the growth of the two organisms in symbiosis was necessary to produce the type of lesion in question. They were not firmly convinced, however, that this was the etiologic factor in the production of the ulcer in their patient.

Shipley⁵ has recently reported a progressive gangrenous ulceration of the abdominal wall of a patient

following drainage of an abscess in the appendical region. A nonhemolytic streptococcus was isolated from the pus. A similar case has been reported by Mayeda,⁶ from which an organism resembling the diphtheria bacillus was isolated.

In the six cases reported, including our own, there are several points in common. All except one (Christopher's case) occurred on the abdominal wall following an appendectomy with drainage, or drainage of an abscess in that region. In Christopher's patient there might possibly have been some kind of a connection between the intestinal canal and the empyema cavity, since colon bacilli were found in the empyema cavity at one time. All of the ulcers have had the same appearance and have been exceedingly tender and pain-

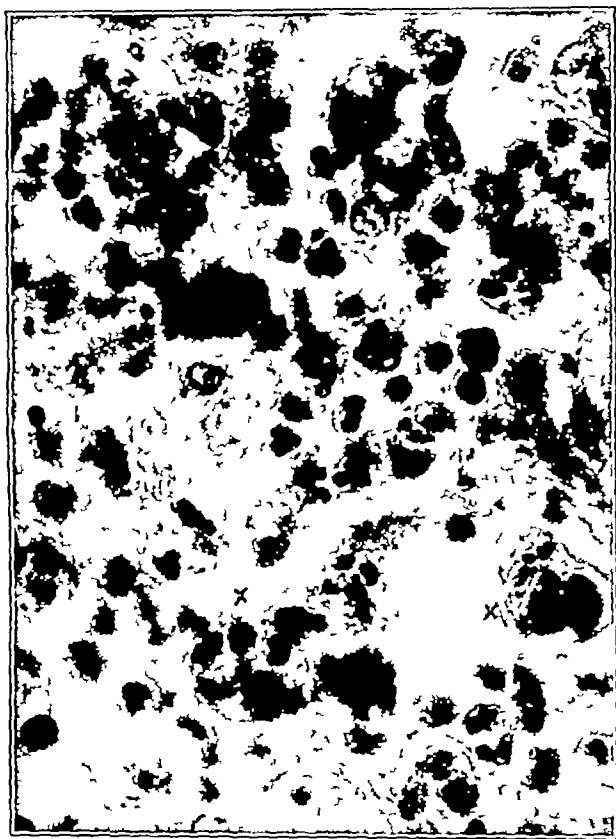


Fig. 4—Higher power magnification of organisms found in the same section as figure 3 but from a different area. X marks location of two organisms in focus.

ful. So far as we were able to determine, all of the patients survived, but only after there had been a radical excision of the actively growing edge of the ulcer. Various types of treatment, including hot wet packs, irrigation with surgical solution of chlorinated soda, potassium permanganate packs, sunlight, ultraviolet rays, infra-red rays and roentgen therapy, have been tried in the cases reviewed, but without any appreciable improvement. In our case, we felt that x-ray therapy was helpful, but Dr. Sherwood Moore, who gave it, was not so optimistic. Gauze packs soaked with emetine were used in the treatment of our patient, but improvement could not be seen.

From the description of the cases reviewed, including our own, it seems logical to conclude that the etiologic factor is the same in all of them, namely, an amebic

2. Cullen, T. S. A Progressively Enlarging Ulcer of the Abdominal Wall Involving the Skin and Fat, Following Drainage of an Abdominal Abscess Apparently of Appendiceal Origin. *Surg. Gynec. Obst.* 38: 579 (May) 1924.

3. Christopher, F. Severe Spreading Carbuncular Infection of Chest Wall Following Rib Resection under Local Anesthesia. *S. Clin. North America* 4: 793 (June) 1924.

4. Brewer, G. E. and Meleney, F. L. Progressive Gangrenous Infection of the Skin and Subcutaneous Tissues Following Operation for Acute Perforative Appendicitis. *Ann. Surg.* 84: 438 (Sept.) 1926.

5. Shipley, A. M. Progressive Gangrenous Ulceration of the Abdominal Wall. *Ann. Surg.* 87: 245 (Feb.) 1928.

6. Mayeda, Tomosuke. Eine seltsame Hautgeschwursbildung nach der Appendektomie. *Deutsche Ztschr. f. Chir.* 199: 350 1926.

organism. We feel that finding the organism in such large numbers in the microscopic section of the actively growing ulcer of our patient, as well as in the pus draining from the ulcer, excludes the possibility of its being a contaminating organism. The fact that emetine produced such striking improvement in the ulcer points also to an amebic origin, despite the fact that emetine failed to eradicate it. Since so many varieties of organisms have been isolated in the cases reviewed, one might conclude also that this type of ulcer is not of coccal or bacillary origin. It was the opinion of Drs. I. Y. Olch, H. A. McCordock, A. J. Kendall and L. D. Thompson, who also studied the sections from our case, that the cell found was a protozoan parasite and could best be classified as an ameba. It is significant that none of the patients reviewed in this report, including our own, were suffering from amebic dysentery.

A brief description of seven additional cases has been found, but insufficient data are given to enable us to

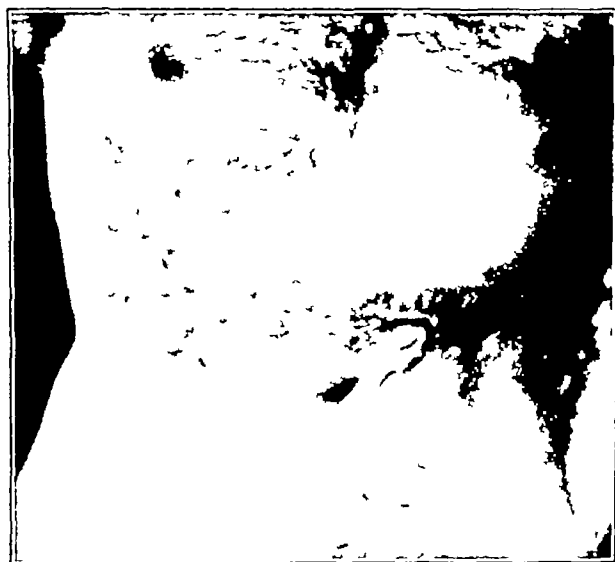


Fig. 5—Appearance of the abdomen after ulcer had healed. Skin grafting was not done. Epithelization and scarring were so rapid that the patient preferred not to run the risk of a recurrence of the process by any manipulation that would be necessary with such a procedure as skin grafting.

conclude with any degree of accuracy that they were of the same nature as our case. The one reported by Alexander⁷ seems identical, especially in the manner of progression, viz., an active invasion of fresh skin for a few days, followed by a period of comparative sluggishness for about ten days. The six other patients were mentioned by Porter (three cases), Moschowitz (two cases) and Clinton (one case) in the discussion of Brewer and Meleney's paper. The ulceration in four of the seven foregoing cases followed operation for appendicitis. A protracted gangrenous ulceration of the chest wall has been observed by J. G. Probst of St. Louis, following an operation for a draining sinus of the breast, but amebas could not be found.

CONCLUSIONS

1. An amebic organism has been found in the pus and microscopic section of an ulcer occurring after an appendectomy with drainage and evidence points to that organism as the etiologic cause.

⁷ Alexander, E. G. Postoperative Spreading Superficial Gangrene. *Ann. Surg.* 84: 461, 1926.

2. The similarity of the lesion in this case to that in the other cases reviewed in this report is so striking that the etiologic factor would appear to be the same.

3. The most efficient and practically the only satisfactory method of treatment is cautery excision of the actively growing part of the ulcer. The excision should extend at least 2 cm. beyond any red areas creeping out from the margin of the ulcer.

4. The possibility that amebas may influence the pathologic process in the appendix in some cases of appendicitis is suggested. Unusual bodies were found in the wall of the appendix in our case but they could not be identified as amebas.

602 South Euclid Avenue—508 North Grand Boulevard

A SPECIALIZED TYPE OF MUSCLE IN THE HUMAN PREGNANT UTERUS

POSSIBLY ANALOGOUS TO THE CONDUCTIVE SYSTEM OF THE HEART*

J. ISIDORE HOFBAUER, M.D.

BALTIMORE

The problem of uterine activity during labor, more particularly as regards the regularity of contraction and relaxation has from time immemorial attracted the attention of the medical profession. Yet if one reviews what is known or conjectured concerning the nervous and hormonal principles that may govern the underlying biologic processes, one is confronted with such bewildering divergence of opinion that it is difficult to form a conception of the pathways for the conveyance of the nervous impulse to the pregnant organ.

Bearing in mind that an appreciation of function presupposes a thorough knowledge of structure, it seemed advisable to approach the object in view by a careful study of the histology of the pregnant uterus, devoting special consideration to its outer layer. It is this stratum which in the past has challenged the attention of several investigators. While there still exists a great deal of controversy concerning the anatomic structure of the wall of the pregnant organ, it is generally agreed that there occurs during pregnancy a remarkable rearrangement of its outer layer, which appears as an external hoodlike structure arching over the fundus and extending into the various ligaments. These observations are mainly the result of the work of Hebe¹, Henle² and Bayer,³ who established the facts in question by employing a special technique of dissection. No substantial research in this field, however, has appeared for a long period.

It is my object in this paper to call attention to certain characteristics of the uterine wall that were established after twenty-six uteri obtained during the various months of pregnancy had been studied. Emphasis is laid on the development in the outer layer of a specialized structure, whose typical microscopic features are in marked contrast with the rest of the uterine muscle and closely resemble the Purkinje system of the heart. Indeed, it is possible to distinguish the fibers of this

* From the Department of Obstetrics, Johns Hopkins University and Hospital.

¹ Hélie. *Recherches sur la disposition des fibres musculaires de l'utérus développées par la grossesse*. Paris, 1864.

² Henle. *Anatomy*, 1873.

³ Bayer in Freund, W. A. *Gynecologische Klinik*, 1885. *Allgemeine Geburtshilfe*, 1903.

specialized system simply on the basis of their microscopic appearance, and it is probable that such histologic modifications must be associated with a corresponding difference in function. While the work here presented is mainly morphologic in character, reference to a number of phenomena established both by clinical obser-

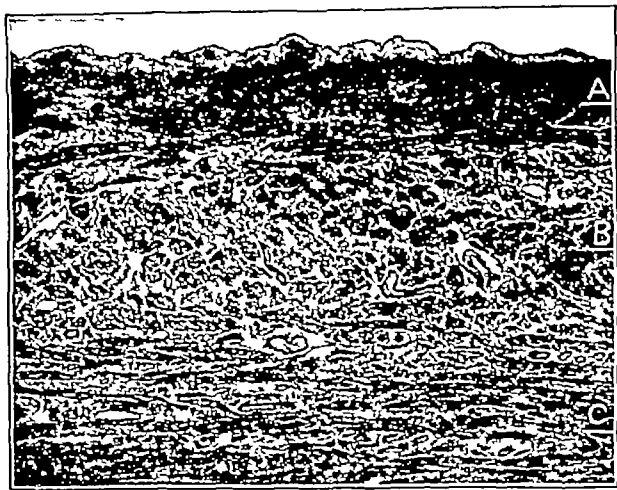


Fig 1—Section through outer half of uterus in the middle of pregnancy reduced from a photomicrograph with a magnification of 40 diameters. A, outermost layer; B, supravascular layer; C, vascular layer. Note the broad connective tissue spaces in layer B.

vation and by biologic research may help to illustrate the physiologic significance of the system under consideration.

For a proper understanding of the topography, it is necessary to present briefly a description of the histologic structure of the wall of the pregnant uterus as shown with low magnification. Beneath the peritoneal covering is seen a thin superficial layer of relatively compact longitudinal fibers, followed by a thicker layer of circular fibers that are spread apart by characteristically arranged connective tissue spaces, which contain many vessels. Internal to this, again, comes a layer of muscles interlacing in both directions, and internal to that comes the bulk of the uterine muscle, which consists of a dense feltlike mass of fibers that extend to the base of the decidua. Hence, it is possible to divide the muscle wall of the pregnant uterus into two parts, a thinner exterior and a thicker interior portion.

At the fundus, the longitudinal superficial layer extends almost over the entire front of the organ but disappears at its sides, where it is connected with the broad ligaments; it is present to a lesser extent on the posterior wall. The development of the longitudinal bundles is most pronounced in the middle third of the anterior uterine wall, all the way from the fundus to beyond the loose attachment of the peritoneum, while on the posterior wall the structure terminates half way between the fundus and the internal os. On the other hand, on the posterior wall of the lower uterine segment there is a superficial band of longitudinally arranged fibers which spreads horizontally to both sides to a varying extent. Figure 1 illustrates the appearance of the two constituents of the superficial uterine layer, the longitudinal band and the circular fibers being separated by broad connective tissue spaces.

The distribution of the specialized system in the outer portion of the pregnant uterus is visible to the naked eye as distinct longitudinal bands, which are arranged mainly in parallel fashion. In transverse and mesial

sections through uteri preserved in Kaiserling's solution, the structure appears as a well defined whitish formation, from 1.5 to 3.5 mm thick, which occupies the entire peripheral zone from the bladder reflection anteriorly to the middle of the posterior wall. There it ends abruptly, to reappear in the region of the lower uterine segment.

When the outer layer of the pregnant uterus was examined microscopically, my attention was first drawn to this tissue by what appeared to be the presence of vacuoles. For the past year I have been investigating this tissue more carefully and have found that it consists of a characteristic structure, conspicuously differentiated by the clear diaphanous character of its specific elements, presenting a histologic picture much like that of Purkinje fibers. In order to determine the histologic character of the tissue, different methods of fixation and staining were employed in preparing the specimens obtained at various stages of pregnancy for microscopic study. Thus, it was found that the technique for its gross demonstration in fixed preparations is simple, while its morphology presents a varying picture according to the fixatives employed. Specimens preserved in solution of formaldehyde, alcohol, or Zenker's fluid do not give such sharp outlines, nor do they show such fine details as when treated by mixtures of osmic acid, acetic acid and potassium bichromate or of chloroform, acetic acid and alcohol. For the cell structure iron hematoxylin and Mallory's method are the best, while sections stained by safranin give better differentiation than eosin preparations.

Figures 3 and 4 illustrate the characteristic features of the structure under consideration. On cross section in figure 3 there is seen a network of cells of very special character. The bulky cells are polyhedral in outline and present an abundant protoplasm, with a central zone enclosing the nuclei. In some specimens this central area is pale, nonstaining and perfectly homogeneous, while the cortical zone, which may be very



Fig 2—Mesial section through upper half of uterus at term showing the white outer zone.

thin or may encroach considerably on the central zone, appears darker because of its granular sarcoplasm. There may also be seen slight differences in the quantity of sarcoplasm in various preparations. Two additional distinguishing features are easily discerned in some of these cells: a zone of condensation running

transversely through the cytoplasm or a partition dividing the cytoplasm into a clear and a darker area. Inter-spread between the elements just described are some elongated cells which exhibit the characteristics of ordinary smooth muscle. Since the cytoplasm of these intercalated cells appears much darker when contrasted with that of the fibers under consideration and, furthermore, as their processes connect veritable frame-

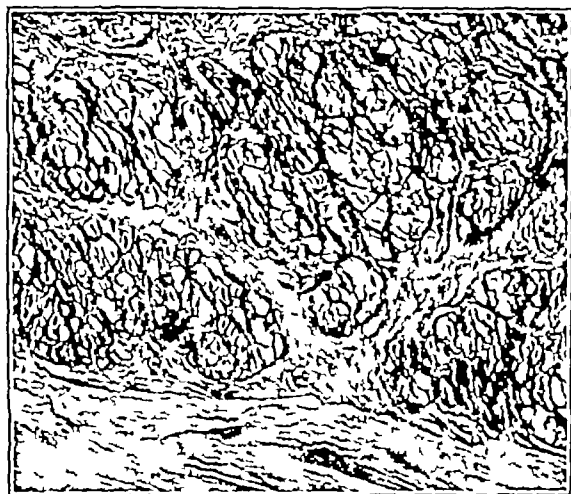


Fig. 3.—Cross section through outermost layer of the same uterus as in figure 1 iron hematoxylin $\times 140$

works are frequently formed resembling a honeycomb in which the clear elements are included. However, there is nothing invariable about this disposition. On the other hand another characteristic of the structure is the presence of a well developed connective tissue sheath, with a few elastic fibers, surrounding the individual bundles, and continuous with it there appear within the fasciculi ramifying bands of connective tissue which are abundantly supplied with delicate vessels and which divide the bundle into several divisions. When the fibers are seen longitudinally (fig. 4) the following characteristic features are noticeable in the first place, from three to five nuclei are present in the axial zone, surrounded by a lucid area, secondly, one end of the fiber ends in a club-shaped process with a homogeneous sarcoplasm. In addition, the plexiform arrangement of the whole structure is worthy of note.

Frozen sections stained with sudan show practically no fat in the fibers, while the occurrence of a few fine fat granules in the perinuclear areas is rather uncommon.

Sections stained by Best's technic reveal the presence of abundant glycogen in the connective tissue spaces, both at the periphery and within the fasciculi, while the glycogen supply in the cells themselves is rather scanty.

Mallory stain emphasizes the distribution of the connective tissue sheaths and gives excellent pictures of the zones of condensation within the individual fibers, as well as some other details of the cell structure.

Iron hematoxylin best brings to light the transverse striation of the fibers, while other stains show little sign of it. Mitotic figures and delicate filaments within the nucleus are also well demonstrated by this stain. The character of the multiple small nuclei is likewise well defined by it when it appears that they are sometimes joined together or sometimes quite distinct from one another, but they always lie in the axis of the cell and are surrounded by an ovoid clear area. True

mitotic divisions may be observed on rare occasions in specimens obtained during the whole course of pregnancy.

The peripheral zone of the cells stains intensely in these preparations and shows granules but no longitudinal striation. A transverse striation of the cytoplasm, on the other hand, appearing as transverse deeply stained lines may be seen, though not invariably in close proximity to the perinuclear zone or situated more toward the rounded end of the cell, but a symmetrical distribution of the striation throughout the entire extent of the cytoplasm occurs only occasionally. The striation in the various cells shows a great deal of variety.

Another particular feature of the elements under consideration is the occurrence of condensation of the cytoplasm, which appears in different parts of the cell as deeply stained disks which occupy a larger or smaller area and are sometimes disposed in a terrace-like arrangement. Our material, however, does not permit any statement as to the correlation between these zones of condensation and the phenomena of localized contraction of the cytoplasm.

The description so far given tends to show that, in all the essential details of their histology, the cells under discussion resemble the Purkinje elements of the conducting system of the heart muscle. Since the cell under consideration is of greater diameter than the ordinary uterine muscle fiber and is conspicuous by its histologic character, its presence in the deeper muscle layers can be ascertained without difficulty. Accordingly, cells of the specialized type may be seen permeating the muscle stratum beneath the vascular layer either as a few isolated elements or more often grouped about the periphery of the muscle strands. Figure 5 may be taken as an illustration of the particulars here described. When cross sections were examined through the entire thickness of the uterine wall, the presence of a thin longitudinal bundle of these cells was distinguishable in the upper two thirds of the uterine body, and interspersed between ordinary muscle strands at the



Fig. 4.—Transverse fibers of the same section reduced from a photomicrograph with a magnification of 370. Note the groups of clear fibers and multiple nuclei.

margin of the uterus. Moreover, microscopic study revealed the occurrence in deeper muscle layers in the lower part of the posterior wall of the uterus of two thin longitudinally arranged bundles, which connect the lower pointed end of the formation on the posterior uterine wall with the macroscopically visible white zone in the lower uterine segment in the region of Douglas' pouch.

Although no special effort has as yet been made to study the nerves, it should be noted that the bundles of the specialized system within the posterior wall of the lower uterine segment and at the margin of the uterus lie in close proximity to nerve fibers. A special study of the relation of the specialized system to the nerves and ganglions will, however, be made in the near future.

On the relations that exist between the specialized system and the ordinary muscle of the human pregnant uterus there is no reference in the literature. When suspended in Locke's solution, strips obtained from the subperitoneal layer of the pregnant uterus strikingly show more frequent and more vigorous contractions than strips taken from other parts of the organ. Likewise, the response to small amounts of pituitary extract is considerably more pronounced in the former. These facts were ascertained in this clinic a few years ago by the extensive work of Sun,⁴ and have been abundantly substantiated in our studies. They are possibly comparable to the statement of Ishihara⁵ that the rhythmic properties of the Purkinje system are higher than those of ventricle fibers. The further fact that, following the incision of the uterine wall at cesarean section, the superficial layer retracts more vigorously than the rest of the muscle, in turn suggests an analogy to the observation made on the heart "that in fresh tissue the Purkinje fibers contract more than the cardiac fibers and dimpling is seen on the fresh cut surface."⁶ Moreover, the physiologic specificity of the structure under consideration is demonstrable in the living organ by its response to pituitary extract. At cesarean section, following the intramuscular administration of this drug, a pale band from 2 to 3 inches wide, composed of parallel fibers, is visible over the anterior surface all the way from the bladder reflexion to the fundus, in its pattern resembling the tenia of the large intestine. The wave of contraction spreads from this band and involves an ever increasing area of the pregnant organ. Synchronous with the first appearance of the tenia in the midline there comes into view an orbicular structure surrounding the insertion of the tubes, as well as a pale zone in the midline of the posterior aspect of the lower uterine segment. These phenomena are most striking when the operation is being performed in spinal anesthesia.

Viewed in this way, the specialized system reveals itself as a particularly susceptible structure. In order to place the results in their proper perspective, it may not be inappropriate to designate the system in point the pacemaker of the parturient uterus.

Impairment of the functional integrity of the specialized system apparently interferes with the coordinated rhythmicity and contractility of the pregnant uterus. The fact that in severe cases of premature separation of the normally implanted placenta, when the uterus fails to contract or to respond to any stimulant employed, these fibers are dissociated by hemorrhage would seem to lend support to my point of view. The further fact that defective development of the specialized system was observed in a specimen obtained from an elderly primipara can also be interpreted as affording presumptive evidence in favor of a correlation of this phenomenon with the incidence of inertia uteri, so frequently seen in such patients. The relationship that exists between some shock conditions and traumatism

of the specialized system will be considered on another occasion.

According to the present conception, it is by means of the conductive bundle of His that the ventricle and auricle of the heart are united functionally. It is through this structure that all cardiac impulses are originated and propagated. In man, the bundle is from 1 to 2 mm in thickness and is conspicuous by the pallor of its fibers.

In view of such a similarity in both their gross anatomic and their histologic features, as well as of the response to certain drugs, I am inclined to assume that the specialized system of the human pregnant uterus may represent an analogue of the His bundle in the heart. Located on the surface of the pregnant organ, it is placed in the most advantageous position for the quick distribution of an impulse to the uterine muscle, the wave spreading from without inward. The ultimate solution of the subject and its proper evaluation, however, must rest on intensive experimentation and



Fig 5—Portion of supravascular layer of pregnant uterus at term, $\times 260$. Note groups of clear fibers in the periphery of ordinary muscle strands.

clinical observation. The histogenesis of the system will likewise call for special study. The evidence at hand tends to show that the development of the fibers is demonstrable from the fourth week of pregnancy, while by the middle of the process the structure has become well established. It is interesting to note that in the premenstrual period the muscle fibers of the outer layer show a definite swelling of their cytoplasm.

Another aspect of the problem is suggested by considering the peculiarities of the development of the uterine muscle during prenatal life. As a result of the work of Werth,⁷ Bayer, and others, it is accepted that superimposed on the main "primary" muscle layer of the fetal uterus there develops during the last few weeks of gestation a new outer muscle stratum, which undergoes a remarkable involution following birth and disappears entirely in the course of the succeeding months. There is strong evidence to indicate the development of this muscle structure from the subserous connective tissue. Hence the term "muscularis serosae" or "paramyometrium." Whatever the factor initiating the

⁴ Sun, Ko Chi. *Bull. Johns Hopkins Hosp.* 36:280 (April) 1925.
⁵ Ishihara, in Lewis, Thomas. *Mechanism and Graphic Registration of the Heart* ed. 3 1925.
⁶ Cowdry, E. V. *Special Cytology* ed. 2 2:857 1928.

⁷ Werth and Graden. *Arch. f. Gynak.* 55:366 1896.

growth of this layer—possibly the anterior lobe of the hypophysis—the transmission of a stimulating substance through the placenta, responsible for the striking parallel in the new formation of tissue elements both in the maternal and the fetal organ, should be considered.

Finally, it may be said that the anatomic and clinical evidence here adduced lends support to the conception that there exists in the outermost layer of the human pregnant uterus a structure closely resembling the conductive bundle of the heart.

URETERAL OBSTRUCTION IN INFANCY

A STUDY OF SEVENTY-FOUR CASES*

MURDITH F. CAMPBELL, MD

Attending Urologist, New York Nursery and Child's Hospital; Assistant Urologic Surgeon, Cancer Institute; and Adjunct Urologic Surgeon, Bellevue Hospital

AND

JOHN D. LYTTLE, MD

Assistant Visiting Physician, Children's Medical Division, Bellevue Hospital, NEW YORK

This article constitutes an outline of a clinicopathologic study of ureteral obstruction in infancy and childhood, a detailed report of which, with follow-up histories, will be presented later. The conclusions in this work were drawn from observation in seventy-four cases of ureteral lesions discovered by autopsy, by urologic examination and, in some instances, by both.

In infants, as in adults, urinary stasis predisposes to and perpetuates infection of the urinary tract. Stasis is predominantly the result of obstruction and in

an incidence of nearly 2 per cent. Infection was demonstrated in four fifths of these by urinalysis or autopsy, allowing one to deduce that approximately one in fifty of all children has some form of ureteral obstruction, which in many cases accounts for an obstruc-



Fig. 2—Dense and tight ureteral stricture at ureterovesical junction in a girl aged 7 months. Urologic examination because of persistent pyuria revealed poor renal function on left side with infection, right side normal. Marked improvement followed ureteral dilation. Most noteworthy was the fact that the weight immediately showed gain, having been stationary for some weeks.

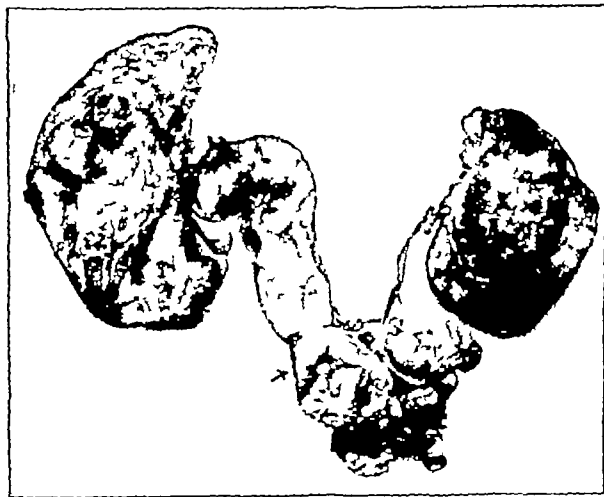


Fig. 1—A rather typical autopsy specimen of ureteral obstruction (congenital stricture) 1.5 cm from bladder in a boy aged 4 months admitted to the hospital for pyuria and dying on the second day, marked hydronephrosis and nephrosis.

infants the majority of urinary obstructions are found along the course of the ureter. A recent autopsy study of 2,420 pediatric cases at Bellevue Hospital revealed gross evidence of ureteral blockage in forty-seven cases,

acute urinary infection and pyuria or not infrequently, recurring attacks of so-called acute pyelitis.

In this paper we are not concerned with the condition commonly called acute "pyelitis" which is probably not an inflammation of the pelvis of the kidney but an infection of the kidney substance. It is almost always accompanied by infection elsewhere (as a tonsillitis or gingivitis) and usually clears up in two or three weeks, no matter what treatment is used. We are dealing here only with those cases of pyuria and other symptoms of renal infection which persist or recur in spite of systematic treatment over a period of six weeks or more.

A certain number of such patients suffer many recurrent acute attacks of renal infection, the acute disease being but an exacerbation of the chronic infection and dependent on the degree of obstruction within the urinary tract. In another group the onset of the urinary infection is insidious, often dating from birth, and in these cases too, the disease remains intractable. Urologic examination reveals obstruction of one type or another in nearly all these cases, and, in the majority, the impediment is found along the course of the ureter.

CHARACTER OF OBSTRUCTIONS

These obstructions may be classified as (1) congenital or acquired or (2) intra-ureteral or extra-ureteral. They will here be considered under the latter heading, since on the intrinsic or extrinsic character of the blockage depends in a large measure the method of

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† From the Department of Pediatrics (Columbia University College of Physicians and Surgeons) and the Departments of Urology, Pathology and Children's Surgery, Bellevue Hospital, Drs. C. H. Smith, A. R. Stevens, Douglas Symmers and C. G. Burdick, directors respectively.

surgical attack. Of the intra-ureteral obstructions, strictures (including valves and blind endings), calculi, kinks, reduplications, abnormal insertions, renal ectopy, ureteral spasm, intra-ureteral cysts and blockage by renal debris must be considered. Aberrant vessels, peri-ureteral sclerotic bands, and extra-ureteral inflammatory or neoplastic masses constitute the majority of the extrinsic group.

INTRA-URETERAL OBSTRUCTION

Strictures—These lesions account for more than half of all ureteral obstructions. Most of those encountered in infancy are congenital and in location show a definite predilection for the ureteral extremes—the pelvic or the vesical insertions. They may, however, occur at any point along the course of the canal, and may be multiple as well as bilateral. In a fifth of our cases we found them bilateral, and in half multiple, but we have never seen more than three strictures in the same ureter. In one instance these lesions were found bilaterally in a seven months' fetus, the upper urinary tract showing marked dilatation but no gross evidence of infection. Certain observers consider these obstructions as mucosal redundancies with valve formation, but, while this condition undoubtedly does exist in some cases, in most instances histologic examination of these lesions reveals marked mural fibrosis. Clinically, however, we are unconcerned with the etiology of the obstruction, renal infection and destruction ensue whatever may be the histologic character of the atresia. At autopsy we have found some cases in which the ureter



Fig 3—Ureteral stricture (presumably congenital) at ureterovesical junction in a girl aged 13 months. Urologic examination revealed infection, dilatation and diminished renal function. Marked improvement followed dilatation of stricture.

ended blindly. Aside from the possibility of surgical pyonephrosis of the excluded kidney, these should cause little clinical concern.

Unquestionably, some strictures of inflammatory origin have their beginning in infancy. In adults, Hunner

has noted an apparent association between ureteral strictures with acute renal infections and focal infectious processes elsewhere in the body. He has explained the acute renal disease on the obstructive basis as an acute congestion of a ureteral stricture or



Fig 4—Two uric acid calculi in lower third of left ureter casting negative shadows in pyelographic mediums in a girl aged 20 months. Scratch obtained on wax bulb catheter; ureteral specimen showed infection and diminished renal function. Calculi were passed following ureteral dilation with two number 5 F catheters passed side by side; there was also a small stone in bladder.

localized infiltration incited by an acute focal process elsewhere, most often in the teeth, tonsils or sinuses. In infants the proof of such an association is even less clear, although it is known that certain renal infections do not clear up until the focal infections (tonsils particularly) are eradicated. The following clinical observation is of interest in connection with this theory.

A boy, aged 7 months, was admitted to Bellevue Hospital with an acute otitis media which was shortly followed by a marked pyuria. At autopsy bilateral acute focal suppurative nephritis and granular ureteritis were found. In the left ureter about 2 cm. below the pelvic junction there was a granulating ulcer about 1 cm. long extending well into the submucosa. The sclerotic healing of such a lesion might well give rise to clinical stricture in later life.

In our series, twenty-five strictures were found at autopsy, and eighteen were demonstrated by urologic examination.

Calculi—Urinary stones are frequently found in children, urinary stasis predisposes to their formation. Most are formed within the kidney pelvis, and they are least often found in the ureter since more than 90 per cent of those which leave the kidney pass to the bladder. In five cases coming to autopsy, ureteral obstruction was found to be due to stone. In two cases showing multiple small calculi in the pelvis, a stone had jammed in the ureteropelvic junction causing marked hydro-pyonephrosis. In two other cases large stones filling

the entire kidney pelvis presented projecting tips which nearly occluded the pelvic outlet. In another instance a large soft stone was quite firmly embedded against a stricture 2 cm. from the bladder. In a further case we demonstrated by urologic examination two stones in the lower ureter of a child, aged 2 years. The history of this case follows, illustrating the use in children of

cases at autopsy and as a secondary lesion in a case clinically. The primary condition was a "cord bladder" with marked upper urinary tract dilatation and infection in a 6 year old girl.

Ureteral Reduplication—Obstruction has been noted at the point of ureteral crossing in some cases of complete ureteral reduplications. We have never observed such a case. Atresia is more commonly noted at the site of bifurcation in cases of incomplete reduplication. In one such case, a girl, aged 5 years, we did not properly interpret our urologic observations, although operation revealed the true condition. The patient complained of right loin pain and pyuria. Examination revealed a "dead" right kidney, urographically a pyonephrosis. We advised nephrectomy, but a reexamination was requested. This revealed quite normal urine, good function and a small, lobulated, smooth-walled pelvis. As repeated lavage failed to improve the condition, the kidney was removed. Examination of the specimen revealed a double kidney, normal above, pyonephrotic below, with an infected, dilated lower ureteral branch.

Abnormal Insertions—Insertion of the ureter high on the kidney pelvis is usually associated with acute angulation at the junction, and not infrequently the lower segment of the orifice assumes a lip or valve formation blocking the pelvic outflow. In this series two such cases are included. At the other extreme insertion into the urethra, the rectum or vagina may be

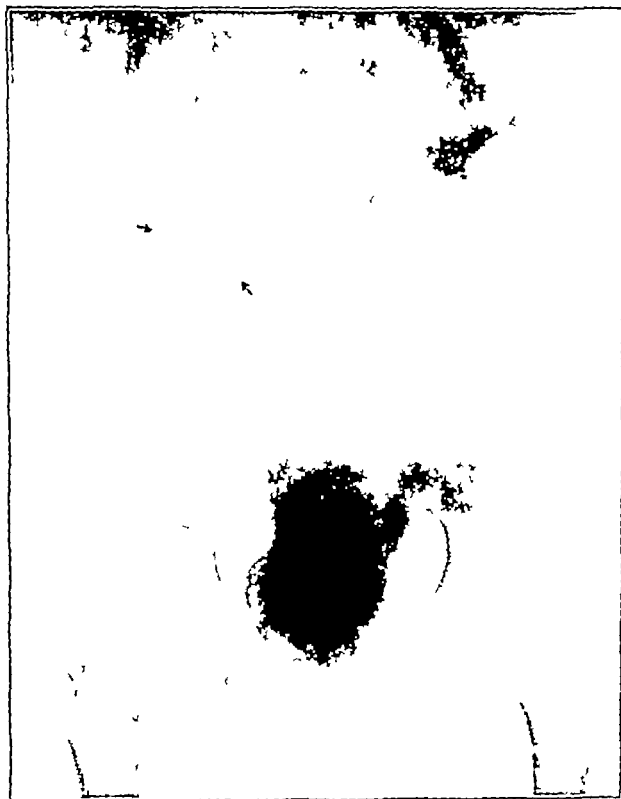


Fig. 5—Ureteral kinks, cord bladder, in a girl aged 6 years with marked dilatation and infection. The kinks here are secondary and probably of no clinical concern. The patient died six months later of uremia.

the same urologic methods of diagnosis and treatment employed in adults.

A girl, aged 20 months, was admitted for pyuria of two months duration. A plain x-ray film was negative. Cystoscopy revealed a small stone in the bladder (estimated 6 mm. diameter). Ureteral specimens revealed marked infection of the left ureter with diminished function (indigo carmine). A pyelogram showed a dilated ureter with two "negative" shadows in the lower left ureter (fig. 4). One week later a wax bulb passed showed stone scratches. The left ureter was dilated with two number 5 F catheters passed side by side. Three weeks later there was no stone in the bladder, no scratch on the wax bulb, and moderate dilatation of the ureter without pyelographic evidence of stone.

In this case we were dealing with three small uric acid stones, all of which were passed. Unfortunately they were lost and no chemical examination was possible.

Kinks—Acute ureteral angulations or kinks are most often associated with renal ptosis or ectopy. They are most often situated near the ureteropelvic junction but may occur at any point. They may be congenital, we know of one instance in mono-ovular twins, in which autopsy revealed sharp S shaped kinkings in the left ureters at precisely the same point. Curiously, there were also identical transduodenal bands. In our series we found kinking associated with ectopic kidney in two



Fig. 6—Ureterospasm frequently and erroneously diagnosed as ureter stricture, in a girl aged 4 years. No obstruction was made out in this case; the apparent constriction was not shown in other films taken at the same time or in subsequent urograms.

associated with obstruction. We have records of autopsies in this type of ectopic insertion, but in none of these cases was obstruction produced. Some observers, however, have reported this type of obstruction.

Ureteral Spasm—Brasch and others have reported numerous cases in which chronic ureteral obstruction

was quite evidently due to ureterospasm. The mechanism of this phenomenon is not understood. Clinically, we observe certain patients showing great unilateral ureteral dilatation without evidence of anatomic obstruction. The lesions are predominantly unilateral and are located chiefly at the ureteropelvic or the ureterovesical sphincters. In our series we had eight such cases, all showing marked unilateral dilatation of the ureter and pelvis without demonstrable sclerotic obstruction. One of these we studied clinically.

Other Types—Intra-ureteral cysts have been noted by some to cause obstruction. We have not seen such instances in children. As a secondary condition, the passage of blood clots or necrotic debris from an ulcerating kidney (tuberculosis, tumor) may temporarily or permanently obstruct the canal. In infancy this condition is rare and of little clinical concern in view of the gravity of the primary condition. At autopsy one patient, a boy, aged 3 days, dying of hemorrhagic disease of the new-born, showed both lower ureters firmly plugged with blood clots and moderate dilatation above.

EXTRA-URETERAL OBSTRUCTIONS

Aberrant Vessels—These most frequently pass from the lower renal pole to the aorta or vena cava, although instances of midureteral and lower ureteral obstruction by anomalous vascularization have recently been reported. In the latter group offshoots of the iliacs, particularly the uterine arteries, have been described. In most instances of obstruction near the pelvic junction, the renal ptosis associated with the more or less slowly forming hydronephrosis aids in accentuating the obstruction. It is believed by many that, could the kidney be kept in its normal position, these vessels would cause little or no obstruction. Apparently, however, the renal enlargement and descent begins rather early in childhood. During the past two years we have seen seven such cases at operation—one in a child of 6 years—and in four of these loin pain had been present since early childhood. They had noted also that great pressure over the loin gave temporary relief—the result of the emptying of a tense hydronephrotic sac.

In this series we are reporting two cases of this lesion, one found at autopsy and the other in a girl, aged 6 years. In the latter case the diagnosis was made by pyelogram (fig 7) and proved by nephrectomy. The clinical course of this patient is of interest in that she had complained of right loin pain, pyuria and intermittent colics since earliest recollection. At the age of 3 an appendectomy had been performed during an acute attack. An extensive search of the literature would indicate that she also represents the youngest recorded case of this type of lesion to be diagnosed urologically and treated surgically.

Periureteral Sclerosis—This may assume the form of bands of adhesions as has been observed by some, or, as in a case of ours, may be due to scarring at the point of intersection of the vas deferens and the ureter. Dense obstruction at this point was determined at autopsy in a patient 5 months of age. Other evidence of deferentitis was not observed.

Extra-Ureteral Masses—These are chiefly the result of inflammation or new growth. In a case of the latter type which we examined recently, in a boy, aged 4 years, the tumor mass so filled the abdomen that a catheter could be passed only 4 cm. A pyelogram revealed the neoplastic character of the ureteral compression.

Large inflammatory masses along the course of the ureter may likewise cause compression. Of interest is

the following case in which a relatively small extra-ureteral lesion caused obstruction. A boy, aged 4 years, dead of lymphatic leukemia, showed acute ureteral obstruction with marked upper dilatation due to an enlarged lymph gland compressing the ureter. Of this group of extrinsic lesions aberrant vessels are probably of greatest clinical interest and importance.

SYMPTOMATOLOGY

The clinical picture is varied and is quite apt to be misleading. Lumbar pain, loin ache, renal colic, hematuria, dysuria and frequency are uncommon or are, at any rate, symptoms difficult to elicit and evaluate in children. Commonly the clinical picture is that of so-called acute pyelitis complicated by an upper respiratory infection or a gastro-intestinal upset. Without



Fig 7—Hydropyonephrosis secondary to aberrant vessel obstruction of ureter. Functionless kidney in a girl aged 6 years. Nephrectomy was performed. Course of aberrant vessel indicated by arrows and by absence of pyelographic medium at this point.

repeated urinalysis, a diagnosis cannot be made. From the history, onset and clinical observations, one cannot differentiate this condition from early pneumonia, meningitis, encephalitis, otitis, influenza or any of the acute infections of childhood.

Pyuria is the most constant observation, but one must remember that in cases of acute and chronic disease occasional specimens of urine may be clear or show only albumin. This is commonly explained as due to a block in the ureter. The significant thing is that after the acute condition is cleared up the pyuria persists or recurs.

In the presence of marked obstruction, palpation may reveal a large renal mass. Acute loin tenderness may also be noted. In most of the infants examined, however, diagnostic signs other than pyuria are wanting.

PATHOLOGY

Pathologically, these obstructions give rise to dilatation and infection of the structures above the lesion, and thus often leads to total destruction of the kidney. There may also be some inflammatory dilatation of the ureter below the block. We have seen infant ureters dilated to the size of the colon with only a thin rim of scar left to represent the renal parenchyma. The kidney which we removed from the 6 year old patient with aberrant vessel obstruction was fully eight times the normal size, hydropyonephrotic, and quite destroyed except at the polar extremes. Prognosis depends in

Ureteral Obstruction in Infancy Seventy Four Cases *

Ages of Patients	Number										
Under 3 months	17										
From 3 to 6 months	13										
From 6 to 11 months	10										
From 1 to 2 years	9										
From 2 to 4 years	10										
From 4 to 6 years	8										
Over 6 years	8										
	71										
	Determined by				Side Involved			Location			
	Autopsy	Urologic Examination	Total	Right	Left	Bilateral	Uretero-vesical Junction	Uretero-pelvic Junction	Body		
Intra ureteral											
Stricture	23	15	47	19	16	8	31	13	6		
Calculi											
Ureteropelvic junction	2		2	2				2			
Filling pelvis and blocking ureter	2		2	2							
In lower ureter	1	1	2	1	1					2	
Kink		1	1		1					1	
Reduplication	1	1	2	2							
Idiopathic (neurogenic)	1	1	2		4						
Tumor	1	1	2	1	2					3	
Tuberculosis	1	1	2	1	1			2			
Hemorrhagic disease of new born, clots firmly plugging ureter	1		1			1	1				
Extra ureteral											
Aberrant vessels	1	1	2	2					2		
Abnormal ureteral insertion	2		2	1	1						
Renal ectopia	2		2	2							
Enlarged lymph node	1		1	1							
Obstruction by vas deferens crossing	1		1	1							
	49	25	74	39	26	9	32	19	12		

Ages range from seven month fetus to 6 years over 80 per cent were under 2 years

large measure on the degree of renal injury, and the treatment is directed toward the prevention of further renal injury

METHODS OF UROLOGIC DIAGNOSIS AND TREATMENT

The methods of diagnosis and treatment are fundamentally the same whether the patient is an infant or an adult. Localization and obliteration of the infection is the urologic problem. The most accurate of our diagnostic data are obtained by cystography, cystoscopy, ureteral catheterization, renal function tests and pyelography. To date our youngest patient to be examined cystoscopically was a boy 10 weeks of age. Instrumentation and the postexamination period were uneventful.

We do not consider instrumentation in infants until they have resisted intensive medical treatment for from four to eight weeks. Persistent pyuria is the indication in most cases. To us hematuria (excepting hemorrhagic nephritis), colic or tumor indicates immediate investigation. Before instrumentation is attempted, certain data are obtained. A plain x-ray film to rule out stone

or spina bifida, a cystogram, one or more phenolsulphonphthalein tests, blood urea and creatinine estimations, blood pressure records and cultures of the urine are routine measures. With these data in hand, one can proceed more intelligently and with less jeopardy to the patient.

Anesthesia is or is not used according to the age of the child and its temperament. We have carried out complete urologic examinations, including pyelography, in 6 year old girls without anesthesia, if the patient is hurt, instrumentation is impossible. We prefer the open drop method and often need less than an ounce of ether, stopping the anesthetic as soon as the instrument is passed. The quiet breathing that follows allows more satisfactory examination of the bladder. Catheterization, the collection of ureteral specimens for chemical, bacteriologic (including culture) and microscopic examination, intravenous dye function tests (indigo carmine is our preference) and pyelography complete the examination. By the time the patient is ready for pyelography, he is well out of the anesthetic and is acutely reactive to renal distention. We rarely perform bilateral pyelography and do not perform any pyelography on the anesthetized child. Wax bulb catheters for stone scratch or other bulbs for stricture detection may be passed as in adults. By confining our examinations to those patients presenting the aforementioned indications, we have found gross urinary lesions in more than 95 per cent of the patients that were studied.

When due to ureteral obstruction, urinary infection can usually be cured by obliteration of that obstruction. Strictures may be dilated by passing two or more small (number 5 F) catheters up the ureter side by side. We perform pelvic lavage with 0.2 per cent silver nitrate but feel that the satisfactory results we have been able to obtain are due to dilatation rather than to the drug. In one case, a girl, aged 19 months, we performed bilateral ureteral incision through a cystotomy incision, the indication being dense strictures of the intramural ureter with bilateral upper urinary tract dilatation and infection. (We were repeatedly unable to pass catheters larger than number 4 F.) Nephrectomy was performed in two cases for pyonephrosis secondary to obstruction (aberrant vessel, ureteral reduplication). In short, the surgical indications are identical with those in adults and, on the whole, these infants withstand instrumentation far better than do adults. Postinstrumental reactions are fewer and less severe. Because of the greatly diminished caliber of the instruments used in children, it is impossible to carry out certain procedures commonplace in adults (particularly ureteral dilation with large bougies).

SUMMARY

Approximately 2 per cent of infants suffer from ureteral obstruction which predisposes to infection and renal destruction. That pyuria which resists systematic therapy for more than four weeks indicates complete urologic investigation is our emphatic opinion. With this as our criterion, we have disclosed gross urinary tract disease in more than 95 per cent of the patients whom we examined. The same urologic, diagnostic and therapeutic procedure followed in adults should be employed for children and infants except when rendered impossible by the small caliber of the instruments.

33 East Sixty-Eighth Street

ABSTRACT OF DISCUSSION

DR. HENRY F. HELMHOLZ, Rochester, Minn. We are greatly indebted to the authors for bringing this important matter to our attention again, because it is the pediatrician who must discover these cases before destruction of renal substance has progressed to such a point that therapeutic procedures of any kind are of practically no value. It is rather interesting in going over the textbooks on pediatrics to notice that in 1894 the pyelitis that was known in infancy and childhood was practically all of the type described so well this morning. Only cases with urinary anomalies are supposed to lead to urinary infection. The early textbooks considered the various anomalies, stones and other conditions as the cause of urinary infection. The advent of Escherich's paper on colicystitis completely changed the conception of pyelitis, and from that time on the pediatrician considered pyelitis a relatively insignificant infection in which the mortality rate was low and from which the patient recovered with or without treatment. Dr. Meyer of Berlin believes that plenty of water and possibly a little sugar is all that is needed to clear up these cases of pyelitis. During this second period, therefore, infections of the urinary tract were considered more or less insignificant. Now the pendulum is swinging the other way. It is evident that there are rather benign infections of the urinary tract which are readily cured and also infections associated with urinary anomalies that are of grave danger to the life of the individual. As pediatricians it is our duty to discover these cases as early as possible. Unfortunately, if we rely on the presence of infection only to make a diagnosis, we are going to be late in discovering many of these anomalies. We should call in the urologist in every case of pyelitis in which the condition does not clear up after from four to six weeks of intensive treatment. If there is blood in the urine not explained by nephritis, if there is a palpable mass in the kidney or bladder region, or if there is colic the urologist should be called immediately. In this way we should solve the problem of this second group of infections.

DR. B. C. CORBUS, Chicago. The urologist is often taxed to the utmost in making a diagnosis. It has been necessary to use anesthesia in order to examine children cystoscopically. This is often followed by nausea, vomiting and a delay in the excretion of urine which makes it impossible to obtain kidney function tests. During the last year I have repeatedly examined little girls as old as 4 years by using $\frac{1}{16}$ grain (7 mg.) of pantopium hydrochloricum (pantopon) hypodermically; those around 6 years were given $\frac{1}{16}$ grain (11 mg.), and those over 9 years $\frac{1}{8}$ grain (21 mg.). Cocaine, 0.5 per cent, and sodium bicarbonate were used locally in the urethra. In no instance was any toxic effect noticed. From a close survey of the case reports and autopsy records published, it has been my impression that many children that present at birth bilateral or unilateral congenital obstruction above the ureteral ostia die within the first year. This observation has been confirmed by Dr. Bilger's splendid anatomic specimens. I believe that acquired stricture of the ureter in children does exist but it does not often manifest itself before the fifth year. In my opinion, chronic urinary infection in children who survive after the first few years of life is many times due to obstruction in the urethra either anterior or posterior, or at the vesical neck. With the present method of cystoscopic examination it is impossible to visualize the posterior urethra in these children, although one can get a fair view of the internal urethral orifice. The classification of the different congenital anomalies that cause urethral obstruction is not obtained by cystoscopic examination, as one would perhaps believe from the remarks of some of our confrères, but, in a large measure, from postmortem observations. I think that vesical neck obstruction plays a larger role in chronic urinary infection. To me the one thing we should do before we attempt cystoscopy is cystography. Often this will reveal a great deal and a determination of the residual urine and the passing of a bougie a boule will many times completely clarify the diagnosis. In all chronic urinary infections the bladder neck and posterior urethra should be examined thoroughly before the upper urinary tract is manipulated.

DR. A. E. GOLDSTEIN, Baltimore. Several years ago in autopsy examinations I found numerous lesions of the urinary tract in both children and adults. Children with fever, nausea

and vomiting have more than just gastro intestinal conditions, as they frequently are diagnosed. From a urologic standpoint, or from the standpoint of the urologist, the presence of blood and pus in the urine is of rather great consequence. I am of the opinion that pediatricians do not pay sufficient attention to pus, blood or albumin in the urine. Even in cases in which the urine is normal, or rather in which there is no pus or blood in the urine, I have found frequent instances of urologic conditions, comprising diseases of the kidney, bladder or ureter, as has been demonstrated here today. The one thing that is clear is that the lesions that exist in children are the same as those that exist in the adult. Therefore, why should they not be sought for earlier, long before uremic coma occurs? In many instances these children can be saved. Several fallacies exist among pediatricians. First, there is the theory that children cannot be examined cystoscopically. I have used the cystoscope in infants as young as 8 days, and in children up to 16 years of age. Second, there is the idea that the treatment of pyelitis in children should consist of forcing fluids or giving methenamine or hexylresorcinol. In my opinion, both these drugs are of no value whatever without the assistance of cystoscopic treatment. The third fallacy is that all children with nausea, vomiting and fever have intestinal disturbances. Frequently the nausea, vomiting and fever are merely the early stages of some urinary condition. What we need in these children, first, is an early urologic examination. Secondly after the diagnosis has been made, they need good drainage irrespective of whether the lesion is in the kidney, ureter or bladder.

DR. FRANCIS H. REDEWILL, San Francisco. If we can prevent these conditions we will be more than interested. Osborn and Mendel have shown that if the vitamin A content of food is decreased calculi and ulcers form in the bladder. McCarrison found that if persons were given milk, fruits and nuts and no meats or white breads, they did not have ulcers or any other pathologic condition in any part of their bodies. He gave thirty-six monkeys nothing but polished rice and white bread, and they developed ulcerated conditions of the duodenum, stomach and bladder, and calculi. On the other hand, when he changed the diet and pushed vitamin A, he cleared up those conditions. Besides that we noticed that duodenal ulcers can be cleared up by injecting parathyroid extract. In my work with bladder conditions, I injected parathyroid and used these vitamins in clearing up some very pernicious conditions of the bladder. Dr. Bidding of San Francisco told me when I found that I could not clean up these conditions by pushing vitamin A alone that a condition of symbiosis exists. One has to give vitamins A, B, C, D and E, as each vitamin acts on the other. I really believe that these congenital conditions can be corrected and prevented by feeding the mother the proper diet and parathyroid. The treatment can be regulated by blood calcium tests. I believe that in the next few years the test for blood calcium will become as important as the test for blood urea.

DR. W. F. BRAASCH, Rochester, Minn. I wish to call attention to a factor in the interpretation of the urograms accompanying chronic infection of the kidney and ureter which has been overlooked, namely that dilatation of the ureter and pelvis is not necessarily caused by obstruction but may be the result of atony. This atonic dilatation may be caused by diffuse infection in the ureteral wall with consequent diminution of peristalsis and atony. It is open to question whether any amount of dilating would be of any help in this condition. It probably explains why in many of the cases of chronic renal infection in children, as well as in adults, improvement has not been shown following ureteral dilation. I would also say that not all urologists are entirely agreed that ureteral dilation is indicated as a routine treatment of pyelonephritis in children. I do not believe that every child who has infected kidneys should be treated cystoscopically. The great majority of these patients will get well with the aid of the time honored and modern therapeutic methods that pediatricians usually employ. In only the occasional case that does not clear up and in which there is definite evidence of ureteral obstruction are we justified in using cystoscopic methods of treatment. No one can deny that actual stricture of the ureter does occasionally occur in these cases, and when it is present dilation is frequently of much value. When atonic dilatation is present, dilating the ureter will not accomplish much.

DR WILLIAM E. STEVENS, San Francisco It is gratifying to observe the increasing interest of pediatricians in pathologic conditions of the urinary tract during infancy and childhood. Urinary tract disease was present in 232 or about 3 per cent of the last 7,447 cases seen in the pediatric department of Stanford University School of Medicine. A large number of these patients were admitted simply because they required circumcision or tonsillectomy. About 30 per cent of these 232 patients suffered from a colon bacillus pyelitis. One half were cured by conservative treatment. Following cystoscopy, catheterization of the ureters and irrigation of the renal pelvis with silver nitrate solution the urine became microscopically negative in almost 100 per cent and culturally negative in about 80 per cent of the uncomplicated cases of pyelitis. In the investigation of the urinary tract the urethra should not be overlooked. Posterior urethral valves in males and strictures of the urethra in both male and female children are not uncommon. The latter occur more often at the external urethral meatus. In the case of a child 5 weeks old who died the autopsy revealed hydronephrosis with destruction of the right kidney, hypertrophy and subacute pyelonephritis of the left kidney, congenital malformation of the ureters, lengthening and dilatation, stricture at the left ureteral orifice, acute cystitis, acute urethritis, left hydrocele, atrophy of the left testis, left inguinal hernia, septicemia, *B. coli* infection and petechial hemorrhages in the skin. This case is unique not only because of the early age at which symptoms of urinary tract disease appeared but also because of the multiplicity of congenital lesions. Age is no longer a contraindication to cystoscopy, ureteral catheterization, functional kidney tests or even pyelo ureterography.

DR CLIFFORD SWEET, Oakland, Calif. I am convinced that healthy children have infections of the urinary tract many times perhaps without any harm. I agree with those who say that urinary tracts should be studied carefully if an infection does not clear up within a reasonable time but there is another point of importance and that is a history as to whether or not this urinary tract infection has found serious lodgment more than once. The mere fact that one can clear up a urinary tract infection by medical treatment and by improving the general condition of the child is no proof that there is not some abnormality in that urinary tract. Periodic examinations of the urine should be made. If I did not misunderstand Dr. Campbell he said that a child of 6 years was the youngest in whom a congenital megalo ureter and complete destruction of the kidney with surgical removal had been recorded. My colleague, Dr. Meads, removed the kidney and a congenital megalo ureter from a girl, aged 23 months. This child had been under our care since birth. She had had repeated attacks of fever with pus and bacteria in the urine. Several attacks had followed upper respiratory tract infection. The last and most severe attack followed whooping cough and otitis media. She had a very large congenital megalo ureter with a large hydronephrosis on the affected side. That kidney, being no longer of value, was removed. The mere presence of pus in the urine during acute illness means little. When pus is found during acute illness the urine should be examined several times, and especially after the acute infection has cleared up in order to determine whether or not it is of only passing consequence.

DR MEREDITH F. CAMIBELL, New York. Regarding the frequency of urinary tract anomalies in the series of autopsies which we studied at Bellevue, namely, 2,420 cases, we found that 176 or a little more than 7 per cent showed urologic anomalies. Some of these showed more than one type of anomaly and of these, thirty one had additional anomalies in other parts of the body, such as cardiovascular lesions and diaphragmatic hernia. When we first started this work at Bellevue, a few pediatricians held up their hands in horror because we were using the cystoscope in babies. However, the large number of pathologic urologic conditions found and the striking clinical results obtained have so interested the pediatric department in the work that for the next year Columbia University has assigned two fellows to work with us in the study of urinary tract infections. Before we began this work intensively, we seldom examined more than two babies a year urologically. At the present time we are examining from six to ten a week. These babies stand instrumentation far better than adults do. Their postinstrumental reactions are less

severe, as a whole and they are also fewer. We have now made more than 500 urologic examinations in children, and we have no cause to hesitate in continuing these procedures. We have tried giving chloral by rectum instead of ether (open drop) anesthesia but have not been able to get the required anesthesia. We are heartily in record with what Dr. Braasch said. In our series we have nine cases that very definitely fall into his classification of atonic dilatation without demonstrable organic obstruction. The cases in which we do instrumentation and ureteral dilation are only those in which we are able to demonstrate a definite obstruction that is, all of these cases have shown marked resistance to the passage of a number 4 catheter. A number 4 catheter is $1\frac{1}{2}$ mm., which is very small. Such a catheter may be passed up the normal infant ureter without difficulty. In answer to Dr. Sweet, the patient in whom nephrectomy was performed was a child who had an aberrant renal vessel. I believe that a case has been reported in the literature of nephrectomy in a child aged 23 days, but this was because of an acute infection not an aberrant vessel. We do not use the cystoscope in every patient who has pyelitis, as relatively few have pyuria that resists medical treatment for from four to six weeks.

YELLOW FEVER VIRUS

TRANSMISSION OF BRAZILIAN STRAINS TO MACACUS
RHESUS AND MACACUS CYNOMOLGUS
PRELIMINARY REPORT*

HENRIQUE DE BEAUREPAIRE ARAGÃO, M.D.
RIO DE JANEIRO, BRAZIL

The observations of Stokes, Bauer and Hudson¹ that *Macacus rhesus* can be easily inoculated with the virus of yellow fever in Africa and that this disease is not due to *Leptospira icteroides* Noguchi have stimulated renewed interest in research on yellow fever in America, where most of the classic studies on this disease have been made.

The occurrence in June 1928, at Rio de Janeiro of several cases of yellow fever presented the opportunity for the experiments reported here.

Only four *Macacus rhesus* monkeys were available in Rio de Janeiro when the first cases of yellow fever appeared. One of these (monkey 1) was inoculated with 2 cc. of a culture of *Leptospira icteroides*, Palmeras strain, isolated by Noguchi and his co-workers,² the other three (monkeys 3, 4 and 5) were inoculated with blood drawn from patients with yellow fever two and one-half, three and three and one-half days, respectively, after the onset of the disease. These four animals all remained well.

Another lot of four monkeys of the same species was obtained from São Paulo, and these, together with one *Macacus cynomolgus* obtained in Rio de Janeiro, were

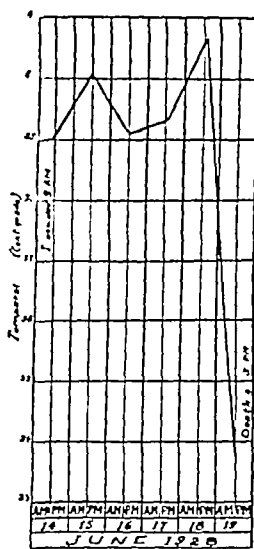


Chart 1—Temperature chart of monkey 8 (*Macacus rhesus*)

* From the Oswaldo Cruz Institute.
1 Stokes A, Bauer J H and Hudson N P. The Experimental Transmission of Yellow Fever to Laboratory Animals. *Am. J. Trop. Med.* 8: 101-164 (March) 1928.

2 Noguchi, Hideyo, Miller, H R, Torres O, Silva F, Martins H, Ribeiro dos Santos A, Vianna G and Biao M. Experimental Studies of Yellow Fever in Northern Brazil. Monograph 20. Rockefeller Institute of Medical Research August 1924.

used for further experiments. Definitely positive results have thus far been obtained with the *Macacus cynomolgus* (monkey 7) and one with *Macacus rhesus* (monkey 8), while, at the time of writing, the other animals are still under observation.

Monkey 8 was inoculated June 14, with the blood of two patients with benign but typical yellow fever (D A and N M). D A was bled forty-two hours and N M twenty-nine hours after the onset of the disease. Five cubic centimeters of blood was given intramuscularly and 5 cc. intraperitoneally. The temperature of the animal rose to 40.6 C on the fourth day after inoculation, followed by collapse, subnormal temperature, and death on the fifth day. The daily temperature of this animal is given in chart 1.

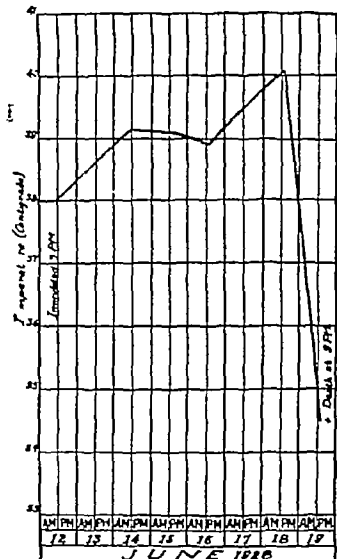


Chart 2—Temperature chart of monkey 7 (*Macacus cynomolgus*)

Autopsy did not show any definite icterus externally. There were no large hemorrhages, the stomach contained a small amount of dark mucoid material giving positive guaiac reaction. The lungs were normal, the heart was pale and flabby. The liver was mottled and pale yellow throughout, except for two small areas on the anterosuperior surface of the left lobe which were somewhat congested; the lobular markings were distinct. The spleen was somewhat enlarged but otherwise normal in appearance, the kidneys were slightly swollen, smooth and somewhat icteric.

Microscopically, the liver showed a heavy deposit of fat uniformly distributed throughout the lobules; there was congestion and vacuolization of hepatic cells, and extensive necrosis, limited mostly to the midzone and not involving the cells immediately surrounding the portal spaces. The kidneys presented lesions of mild acute nephritis, cloudy swelling with desquamation, and necrosis of the epithelium and hyaline casts, especially in the straight tubules.

Monkey 7 (*Macacus cynomolgus*) was inoculated, June 12, with blood from two patients with mild typical cases of yellow fever (J K. and N M). J K. was bled fifty-nine, and N M twenty-nine, hours after the onset of the disease. It will be noted that blood from the latter patient was also used for the inoculation of monkey 8 as already described. Five cubic centimeters was given intramuscularly and 5 cc. intraperitoneally. On the fifth day after inoculation, this animal showed slight fever and loss of appetite, on the sixth day the temperature registered 40 C., followed by subnormal temperature, collapse, and death on the seventh day. The daily temperature of this animal is given in chart 2.

Autopsy did not show definite icterus externally. The lungs showed slight hyperemia. The heart was pale and flabby. The stomach contained a considerable amount of chocolate brown material. There were petechial hemorrhages in the mucosa of the stomach and duodenum. The contents of the lower part of the small intestine showed a considerable amount of altered blood similar to that found in the stomach and there were petechial hemorrhages in the mucosa. The liver was very pale yellow and, on section, fatty in appearance. The spleen was slightly enlarged but otherwise normal. The kidneys were very pale and jaundiced and apparently somewhat swollen.

Microscopically, the liver showed the typical pathologic changes of yellow fever in the midzone areas, as described by Rocha Lima in man. The kidneys show intense acute nephritis with calcareous and hyaline casts. There was fatty degeneration

and necrosis of the epithelial cells in the straight as well as in the convoluted tubules.

In the blood, organs and urine of infected monkeys, *Leptospira icteroides* could not be demonstrated by direct examination, culture or animal inoculation.

SUMMARY AND CONCLUSIONS

Yellow fever occurring in Brazil has been successfully transmitted to *Macacus rhesus* and also to *Macacus cynomolgus*. The gross and microscopic pathologic changes produced by the South American virus in these animals are fully comparable to the lesions produced by the West African virus in rhesus monkeys, as described by Stokes, Bauer and Hudson¹. Furthermore, comparison of sections of liver from *Macacus rhesus* infected with West African virus, brought to Brazil by Dr. Carlos Chagas from the Pasteur Institute of Paris, has not shown any distinguishing features in the lesions. *Leptospira icteroides* Noguchi was not found in two infected monkeys studied.

Further studies are being undertaken which will be the subject of a future detailed report.

NOTE.—Soon after the preparation of this report the following developments occurred:

Macacus rhesus 9 inoculated with blood from patients J K and R M died on the fifteenth day after inoculation. Autopsy showed lesions typical of yellow fever, liver and kidney hemorrhage into the stomach and intestine, and albuminuria.

Macacus rhesus 35 died six days after exposure to ten *Aedes aegypti* mosquitoes which had fed seventeen days before on patient N M on the second day of illness. Autopsy showed icterus and liver and kidney lesions typical of yellow fever.

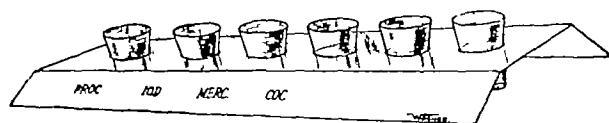
Clinical Notes, Suggestions and New Instruments

SAFETY DRUG RACK FOR THE OPERATING ROOM

SAMUEL IGLAUER, M.D., CINCINNATI

The rack illustrated here is designed to prevent mistakes in the use of drugs in the operating room. Fatal cases are on record¹ in which for example, a cocaine solution has been substituted for a procaine solution.

The rack consists of a single piece of rustless sheet metal bent over in its long axis so as to form a stand which is perforated on top by a series of holes for medicine glasses. The abbreviated names of the drugs commonly employed are stamped



Safety drug rack for use in operating room.

into the front face of the metal frame, and each drug is placed in the container opposite its own name. Thus both the surgeon and the instrument nurse can tell at a glance from which container the drug is being withdrawn. As an additional precaution a toxic solution such as cocaine may be tinged with methylene blue (methylthionine chloride U S P) or fluorescein, or a specially colored medicine glass may be used. Since the rack is made in one piece, it can readily be sterilized by boiling.

707 Race Street

¹ Mayer, Emil. Toxic Effects Following the Use of Local Anesthetics. J. A. M. A. 82: 876 (March 15) 1924.

Special Article

OCCUPATIONAL POISONING IN MANUFACTURE OF LUMINOUS WATCH DIALS

GENERAL REVIEW OF HAZARD CAUSED BY INGESTION OF LUMINOUS PAINT, WITH SPECIAL REFERENCE TO THE NEW JERSEY CASES

HARRISON S. MARTLAND, M.D.
NEWARK, N. J.

(Concluded from page 47.)

SPECIAL SYMPTOMATOLOGY

The Anemias—The deposits in the bones, because of the great insolubility of the sulphates of radioactive substances act as fixed deposits from which they continually emit irritative rays that constantly bombard, day after day, month after month and year after year, the adjacent blood-forming centers producing in time a severe and often fatal anemia.

The nature of the radiation is very important. Approximately 95 per cent of the rays coming from these bone deposits are alpha, the remaining 5 per cent consist of beta and gamma rays.

The alpha rays which consist of the nuclei of helium atoms containing two positive charges (He^{++}), are ejected from the radioactive substances with great force, attaining an initial velocity on leaving the extranuclear electronic field ranging from one-twentieth to about one-twelfth that of light. They represent the fastest mass occupying objects yet known. Lind² says, "The alpha particle is the most potent agent yet known to science and in all probability will remain so as it is hardly conceivable that any means will ever be devised of imparting to ponderable matter a velocity exceeding that at which the alpha particle is expelled from the atomic nucleus." It has great disruptive powers. It decomposes water into hydrogen, oxygen and hydrogen dioxide, and hydrochloric acid into chlorine and hydrogen. These chemical changes are due to the terrific impact of the alpha particle and the atom, as it usually passes through or near the electron system surrounding the nucleus and jerks off one electron. The chemical reaction resulting from this ionization is of the ordinary molecular character. On rare occasions the nucleus of lighter atoms may be squarely struck by the alpha particle, with consequent atomic disruption and the ejection of a high velocity hydrogen atom (H rays).

Biologically the alpha rays are much more destructive to human tissues than either the beta or the gamma rays, the relation being 10,000 to 100 to 1, respectively. Therefore, radioactive elements in such small amounts that beta gamma radiations are almost negligible still produce, through their alpha radiations, intense physiologic effects, if given by mouth or by vein.

In no other previous record of body injury due to radiation has the alpha ray played such a conspicuous part as in these cases, since it ordinarily is easily screened by the radium container or the skin in all forms of external radiation. This is the first time in the history of radiation that the alpha ray has been allowed to play continually on such vital centers as the blood-forming organs. Alpha rays are easily screened and filtered out by even a thickness of paper. In the body they are almost completely absorbed by 1 mm

(one twenty-fifth inch) of body tissues. Because of the proximity of the blood-forming centers to the bones and bone sinusoids of the reticulo-endothelial system, the distance is not too great to prevent the disastrous effects of alpha radiation on these centers. Furthermore, as the charge of the alpha particle is positive and the colloid systems of the erythrocytes are strongly electronegative²⁵ and are permeable only by an anion, the red cells are particularly liable to injury.

In the early fatal cases, the morphology of the blood toward the end was that of a profound leukopenic anemia suggestive of the regenerative type. The color index was often one plus, and a large cell anisocytosis with many microcytes was not infrequent. Occasionally a megaloblast was found. The leukocytes were always diminished in number, a definite leukopenia existing the lymphocyte being the predominant cell. In some cases the polymorphonuclears were practically absent from the peripheral blood. In only one of five fatal cases was there a hemorrhagic diathesis, and in this case the megakaryocytes were undoubtedly affected as the blood platelets were markedly decreased. Petechias which sometimes occurred in the terminal stages were due chiefly to the superadded sepsis. In spite of the resemblance of these early cases to pernicious anemia, there was little or no evidence of the so-called increased hemolysis. The icterus index was normal or low, and the van den Bergh tests were negative. In the cases in which autopsies were performed there was no pronounced increase in hemosiderin deposits in the liver, spleen and kidneys, such as is seen in Addisonian anemia.

As anatomic evidence that the anemias in these early fatal cases were not of the aplastic or regenerative type a dark red marrow was found throughout the femurs in five cases. I specify the marrow of the femur as I believe that this is one of the few locations in which the accurate interpretation of the type of marrow is reliable.

Microscopic examination of paraffin sections from the red marrow of these early cases showed a regeneration of the megaloblastic type. The predominating cell was interpreted as belonging to the erythroblastic series, very numerous normoblasts and megaloblasts being present, many containing little or no hemoglobin. The fatty tissue of the marrow was almost entirely replaced by this hyperplastic marrow. Megakaryocytes were very abundant in most cases. Eosinophils and eosinophilic myelocytes were very numerous.

The occurrence of this apparently hyperplastic marrow was puzzling, since, heretofore, in the report of anemias due to undue exposure to x-ray and gamma radiation from radioactive substances, they have been usually described as distinctly aplastic in type. As in practically none of these cases was necropsy performed or study of the bone marrow made, this conception is purely a clinical one and may not always be substantiated by the facts as we know them.

I had the opportunity of studying the bone marrow sections in these cases with Drs. Edward Krumbhaar and Douglas Symmers. We agreed that there were many cells which could not be identified with certainty. It was impossible to say whether these cells were of erythroblastic or myeloblastic origin, or whether they arose from the undifferentiated metocyte. Oxidase stains in such a dilemma usually are of little value. The fact that the marrow was intensely red would seem to be against the predominating cell being myeloblastic.

in origin. Unfortunately smears from the marrow were not taken, which method is perhaps better than paraffin sections for accurate identification. The question of a myeloblastic marrow which may appear red to the naked eye cannot be lightly dismissed, when it is recalled that cases of undue exposure to radiation in which a hyperproduction instead of a leukopenia occurs have been reported. This has been of sufficient degree to produce a leukemia in several instances. The difficulty in the proper interpretation of bone marrow is well recognized and the development of new chemical stains and the study of the marrow during life in various stages of anemias is greatly needed. In one of Flinn's²² patients, a dial painter who worked in a Connecticut plant and died in New York, the condition of the marrow presented the same general appearance as is seen in chronic benzene poisoning. Pappenheimer,²⁹ who performed the autopsy in this case, told me that the marrow was aplastic.

In the case of von Sochocky, numerous blood examinations made during his last illness never showed the slightest attempt at regeneration, even after the administration of a liver diet. The anemia was definitely aplastic throughout its course, the red cells never showing the anisocytosis and macrocytosis seen in the earlier cases. The latter cases, therefore, appear to resemble more closely those previously described in the literature.

In the excellent review by De Laet²⁷ on the deleterious effects from the professional handling of radioactive substances and γ -rays, he cites the following cases:

Aubertin and Bordet (1909) examined the blood of radiologists and animals after irradiation and found persistent leukopenias often followed by a slight polynucleosis with an eosinophilia.

Vaquez relates the case of a radiographer who died in 1911 of myeloid leukemia.

Mottram (1920) states that a single exposure to gamma rays of radium may produce injury to the blood-forming organs. Persistent exposures may lead to an aplastic anemia and in rare instances to a myelogenous or even lymphatic leukemia.

Mottram and Clark (1920) found in irradiated animals leukopenias alternating with periods showing a leukocytosis.

Mottram (1920) reports three deaths among the personnel of the London Radium Institute from exposure to radium alone. All three patients had leukopenic anemias. No postmortem examinations were made.

Ross (1921) thinks that the gamma rays from radium are more penetrating and cause more injury to the blood-forming organs, particularly the leukoblastic centers, than do the γ -rays.

Caffaratti (1923), in examining the blood of fifty radiologists, found leukopenias which often occurred early and were persistent. Most of the subjects recovered when they withdrew from exposure.

Williams (1923), in a study of the personnel measuring radioactive substances in the U. S. Bureau of Standards, found leukopenias and mild anemias.

Amundsen (1924) found leukopenias in fifteen subjects coming in contact with radioactive substances.

Rud (1924) found leukopenias in six physicians, six nurses, two technicians and three assistants attached to an γ -ray department, due to exposure to γ -rays and radium.

Faber (1924) relates the case of the radiologist Nordenhoff, who died in 1922 from an aplastic anemia.

Carman (1924) reports the case of Dr. Mills, a radiographer, who had an acute appendicitis and lymphatic leukemia.

Weil and Lacassagne (1925) report the death of two workers who were engaged in the separation of thorium X from mesothorium in the same laboratory. One died of an aplastic anemia and the other from myelogenous leukemia.

Weil (1926) described a case of myelogenous leukemia following several injections of thorium X.

Jagic, Liebenrock and Scharz, cited by Jaulin (1927), observed four persons exposed to γ -rays and radioactive substances who died from lymphatic leukemia.

Jaulin (1927) reviews seventeen deaths of subjects coming into contact with radiations.

Brule and Boulin³⁰ (1925) state that only a few cases of pernicious anemia due to radium can be found in the literature, but this severe disease is without doubt more frequent than published reports indicate.

Carman³¹ states that Dominici succumbed to an aplastic anemia due to the habit of carrying packages of radium in his pockets, and that Tiraboschi, an Italian roentgenologist, died of an essential anemia due to exposure to γ -rays.

It is worthy of note in a review of the literature that, with the exception of two cases reported by Weil and Lacassagne and one by Reitter and Martland, no autopsies were performed in any of the cases. To allow the opportunity of collecting accurate data for the future protection of workers in radioactivity to be wasted is quite incomprehensible.

The opinions of different authorities all agree that contact with radioactive substances and γ -rays frequently produces, aside from lesions in the external organs such as the skin, deep and often profound alterations, of which the principal objective symptoms are usually a leukopenia, more rarely a leukemia, an anemia usually of the aplastic type but sometimes with malformations of the red cells suggesting an anemia of the pernicious or regenerative type, a fall in systolic blood pressure and sterility. These alterations may occur very early after the first contact and be mild in character and curable, or, when once established after undue exposure, they may persist even after cessation of the exposure.

Even with the most modern methods of protection, such as proper screens, frequent shifting in work, vacations, and proper ventilation, all workers with γ -rays and radioactive substances are exposed to the deleterious effects of radiation and are potential candidates at any time for the development of a serious and fatal anemia or leukemia. In these anemias, if the leukoblastic centers for the formation of the granulocytes bear the brunt of the injury, sepsis, bone necrosis, endocarditis or septic bronchopneumonia are apt to supervene. If the megakaryocyte is destroyed, the diminution in blood platelets causes the hemorrhagic diathesis to predominate, and hemorrhages from the mucous membranes, bowel, lungs and kidneys occur. If the main injury is to the erythroblastic centers, the symptoms of a profound anemia of the aplastic and sometimes of the pernicious types result. Combinations of all occur.

I am at loss to explain the exact *modus operandi* of the anemias in these cases. They differ in some

30 Brule, M. and Boulin, R. Un cas d'anémie aplastique provoquée par le radium. *Bull. et mem. Soc. méd. d'hop. de Paris* 49: 528 (March 27) 1925.

31 Carman, R. D. and Miller, A. Occupational Hazards of Radiologist. *Radiology* 3: 408-419 (Nov.) 1924.

29 Pappenheimer, A. M. Personal communication to the author.

respects from those already recorded in the literature, and this is probably due to the fact that the alpha particle is the important etiologic factor. Why was a red, regenerating marrow present in these early cases? A discussion of this question demands consideration of recent advances in the study of pernicious anemia.

Although Cohenheim in 1876 considered pernicious anemia as due primarily to a disturbance in blood formation, most writers have looked on the disease as a hemolytic anemia. Their arguments were based on the evidence of increased blood destruction, such as an increased icterus index, positive indirect van den Bergh tests, increased bilirubin derivatives and the presence of increased hemosiderin deposits in the spleen, liver and kidneys. The hyperplastic red marrow found at autopsy was considered an attempt to overcome this increased destruction. This view was considered by most observers in the last ten years as settled and indisputable.

The colossal advance made in the last four years in the understanding of the nature of this disease has been due almost entirely to the work of American investigators. Outstanding research of which American medicine should be proud has been accomplished by McCollum³² on the close resemblance of sprue, pellagra and pernicious anemia and the beneficial effects of liver soup in sprue, by Whipple and Robscheit-Robbins³³ on blood regeneration in severe anemias and the favorable influence of liver, heart and skeletal muscle in the diet, by Minot and Murphy³⁴ on the treatment of pernicious anemia by a liver diet, by Minot, Cohn and others³⁵ on the treatment of the disease by liver fractions, by Perbody³⁶ on the histologic study of the bone marrow in different stages of the disease by using the method of bone marrow puncture devised by Ghedini and elaborating on the observations already made by Zidek, showing that during the remissions the marrow returns to its normal state, and by Castle and Locke³⁷ on the importance of achylia gastrica as preventing the formation of the specific substance which is needed to cause maturation of the erythroblastic centers. Nobel prizes have been awarded for work of less importance.

Haden³⁸ says

We must look on pernicious anemia as due primarily to an inability of the bone marrow to mature red cells resulting from the lack of some specific substance and thus classify it among the deficiency diseases. Tissues other than the liver such as the kidney, can furnish the specific substance, so the deficiency is not necessarily a hepatic one. It is more likely due to a failure of absorption, or an elaboration in the intestinal tract of the specific maturing substance, perhaps resulting from a long existing achlorhydria.

The increased amounts of bilirubin in the blood, tissues and secretions represents not evidence of increased hemolysis but an excess of pigment over what the marrow can use in active stages of the disease.

In the study of the obscure anemias the appearance of the marrow at autopsy can no longer be taken as

conclusive. The marrow is undoubtedly subject to all sorts of terminal revolutions. I have just had the opportunity of observing at autopsy a red marrow of the regenerating type in a man who died from an aplastic anemia due to chronic benzene poisoning. He was a dope man in an artificial leather plant. Two other members of his family who worked in the same plant have died from chronic benzene poisoning. All three had aplastic anemias during life with marked leukopenias, thrombocytopenias and bleeding from the mucous membranes, gums and intestinal tract. Yet from the previous recorded cases of benzene poisoning hardly any one would believe me when I state that this man died from the poisonous effects of benzene.

Is the anemia in these cases of radium poisoning essentially an aplastic one due to destruction of the erythroblastic centers by continual bombardment of irritative rays, particularly alpha? Was there an heroic first attempt in the early cases to produce red cells causing at autopsy a red marrow? Would these early cases, if death had not supervened, finally have become truly aplastic at autopsy as did Pappenheimer's case? All these ideas are purely speculative from our present knowledge.

The Jaw Necroses—What then is the explanation of the extensive necroses in the mandible and often the maxilla seen in the early fatal cases and absent, recovered from or never a prominent symptom in the patients now alive?

In some of these cases this necrosis would precede the appearance of a severe anemia, in other cases it would develop after the anemia had existed for some time. There does not seem to be any connection between the two. In our early work¹ in 1925, we were at a loss to explain the localization of this extensive necrosis in these bones. As the jaw necrosis was the outstanding feature of the early cases our attention was fixed mainly on this symptom and little was paid to changes in the other bones of the skeleton, although an extensive coxa vara was noted in one case. The necrosis of the jaw was at that time thought to be due to local irritative radiation at the portal of entry, produced by clinging particles of radioactive substances on the gums, in the crevices and interstices between the teeth and the bone activated by increased bacterial virulence of the organisms in the mouth caused by the irritative or stimulative effects of small amounts of radiation. These bones were thought to contain a greater amount of radioactivity than did other bones of the skeleton. It was not until I began to see the later cases that I realized that this was not the correct interpretation of the lesion. In the later cases localized pain had occurred in other bones and destructive lesions were demonstrated by the roentgenograms. These lesions were particularly noticeable in bones exposed to trauma, like the scaphoid in the feet (tight shoe), and those carrying considerable weight such as the spine and the heads of the femurs. These lesions so far have never become infected. Autopsies had also shown that the jaw did not contain any more radioactivity than did other bones. In other words, the lesions were quite evenly distributed over the entire skeleton although in each individual bone the distribution while diffuse was distinctly patchy and concentrated in areas. This distribution was demonstrated by photographic methods. Furthermore, histologic studies of bones in which during life there had been localized pain and a roentgenographic appearance unlike that seen in any other disease, showed that the

32 McCollum E. V. *The Newer Knowledge of Nutrition* ed. 2 New York Macmillan Company 1922.

33 Robscheit-Robbins F. S. and Whipple G. H. *Blood Regeneration in Severe Anemia* *Am. J. Physiol.* 72: 408 (May) 1925.

34 Minot G. R. and Murphy W. P. *Treatment of Pernicious Anemia by a Special Diet* *J. A. M. A.* 87: 471 (Aug. 14) 1926.

35 Minot G. R., Cohn E. J., Murphy W. P. and Lawson H. A. *Treatment of Pernicious Anemia with Liver Extract* *Am. J. M. Sc.* 175: 599 (May) 1928.

36 Peabody F. W. *Am. J. Path.* 3: 179 (May) 1927.

37 Castle W. B. and Locke E. A. *Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia* read before the Annual Meeting of the American Society for Clinical Investigation Washington D. C. April 30 1928.

38 Haden R. L. *The Essential Nature of Pernicious Anemia* editorial *J. Lab. & Clin. Med.* 14: 189 (Nov.) 1928.

marrow of these bones was replaced by a gelatinous, myxomatous, cellular loose fibroblastic tissue similar to that described by Ewing³⁹ in bones exposed to heavy external irradiation

From these observations it was more logical to interpret the jaw necrosis as being primarily due to a radiation osteitis similar to that occurring in other bones. Owing to the proximity of the mouth, which is the dirtiest part of the body, and to the fact that the jaw bones have embedded in them the teeth, which act as foreign bodies, and because of the common occurrence of pyorrhea and root abscesses, the jaws are easily subjected to a superadded bacterial infection, in these cases in bones already the seat of a previous disease. The slightest dental intervention may start an intractable necrosis of these bones.

The Late Cases—In the patients now living, the severe anemias and extensive jaw necroses which were the outstanding features of the early cases have not occurred up to the present time. The disease is now present in a milder form, undoubtedly as the result of the natural uninfluenceable decay of the mesothorium, which formed the largest percentage of the original deposits. There is a consequent diminution in the amount of irritative radiation. The main symptoms now are crippling bone lesions, the result of radiation osteitis. While it appears that many of these girls will escape a fatal outcome, one cannot be too sure of this, as many show considerable radioactivity at the present time.

DIAGNOSIS DURING LIFE

I have found that the easiest and most reliable method with which to detect the presence of radioactivity in the human body during life in this disease is by utilizing the expiratory air, for the demonstration of either the presence of emanation by means of an electrometer or the presence of the alpha particle by means of scintillation methods. As the total amount of radioactive substances deposited in the entire skeleton is small, ranging from 14 to 180 micrograms in the patients examined at autopsy, and because the human body cannot be rolled up like a ball and placed opposite the electrometer, the detection of these small amounts of radioactivity by the gamma electrometer is not ordinarily possible. This is especially true when it is recalled that only a small amount of the radiation coming from these deposits is due to gamma rays, and that these rays must travel through a considerable amount of flesh before they leave the body, most of them being absorbed in this passage.

METHODS FOR DETECTION OF RADIOACTIVITY

The following, briefly described, are various methods available for the detection of radioactivity in the human body.

Demonstration of Emanation in Expired Air—Working with Conlon and Knief I first demonstrated the presence of emanation in the expired air of these patients on June 16, 1925. The only previous demonstration of emanation in the exhaled air of the human being that I know of was made in 1915 by Seil, Viol and Gordon⁴⁰ after the experimental ingestion of 50 micrograms of radium (element) by mouth. They said "Radium taken into the body is continually disintegrating with the formation of the gaseous radio-

active substance, radium emanation. This gas is carried by the blood to the lungs, where the emanation passes out of the blood by diffusion, and is lost from the body in breathing."

The demonstration of emanation in the expired air is not a difficult one but it should be performed by a physicist well versed in radium work. The subject passes the expired air by means of a rubber tube through a series of moisture absorption bottles containing calcium chloride and sulphuric acid into the ionization chamber of an electrometer for a period of five minutes, the stopcocks on the instrument then being closed. I usually have the subject blow the air through a cylinder containing glass wool or calcium chloride, then through two cylinders containing sulphuric acid, another containing glass wool, and then into the ionization chamber of either an alpha electroscope of the Lind type or, better, an electrometer of the Wulf type. The latter instrument has a silvered quartz thread, is of very small electrostatic capacity, and by the emanation methods can be read quantitatively to 0.001 microcurie.

Readings are then begun. The accompanying table gives the readings of a test made on one of the dial painters.

Demonstration of Emanation in Expiratory Air

Normal leak during test	8.5 subdivisions in 50 minutes
	33.5 subdivisions in 1st 10 minutes
	16.2 subdivisions in 2nd 10 minutes
	15.7 subdivisions in 3rd 10 minutes
	16.6 subdivisions in 4th 10 minutes
	17.7 subdivisions in 5th 10 minutes
Leak during test	99.7 subdivisions in 50 minutes

In this case there was a much greater leakage during the first and second ten minute periods than thereafter. This is due to the fact that the expired air contains a mixture of emanation originating from mesothorium with its decayed products and radium.

The chamber of the electrometer must then be deemanated. This is accomplished by passing air through it by means of a suction pump for a few hours. On account of small amounts of active deposit, the chamber becomes actually contaminated and should not be used for another test until about four days later.

The objection to this test for the examination of a considerable number of workmen is obvious, since it is cumbersome and time consuming.

Determination of Radioactivity by Means of Gamma Electroscope—June 16, 1925, von Sochocky, Conlon and I used a gamma electrometer of the Wulf type to demonstrate penetrative gamma rays coming from the body of one of the girls. This is the first time as far as we know that this test was made on a human being. The instrument was placed 18 inches from the chest of the patient and 9 inches above the level of the bed. Readings showed a normal leak before the test, ten subdivisions an hour, and a total leak during the test of fourteen subdivisions an hour. The difference of four subdivisions an hour was probably caused by penetrative radiations coming from the body of the patient. The girl died a few days later. The increased leakage, however, was small, and was merely a suggestion of the presence of radioactive substances in the body. After autopsy in this case it was estimated that the skeleton contained 180 micrograms of radioactive substances, about 70 per cent being from mesothorium and its decay products.

On account of the superiority of the method of examining the expired air for the presence of emanation

³⁹ Ewing, James. Radiation Osteitis. *Acta Radiol.* 6:399, 1926.
⁴⁰ Seil, H. A., Viol, C. H. and Gordon, M. A. The Elimination of Soluble Radium Salts Taken Intravenously and per Os. *Radium* 6:42 (May) 1915.

The results given in Table X show that heating at 60°C. for one hour causes a greatly increased surface potential, especially at the higher salt concentrations. Northrop and de Kruif (*loc. cit.*) showed that the critical potential for agglutination to occur in the presence of immune serum was in the zone from -13.0 to $+13.0$ millivolts, and later work has tended to narrow rather than broaden this zone. Taking the value of -13.0 millivolts and studying the results obtained in 0.075 N salt, it is evident that heating to 60°C. for one hour causes the vibrios to assume a potential above the critical point, and accordingly makes them less capable of being agglutinated. In the cases of the two Rangoon Rough strains, the results show that even in the living condition these organisms are above the critical value, and the effect of heat is again to cause a large increase in potential difference.

These observations explain at least in part the results of Linton and Seal (1935) who showed that heating vibrios at 60°C. even for half an hour often either reduced or completely abolished their agglutinability. This result is understandable from the data given in Table X.

For the experimental work given in Table XI, the vibrios were taken up in distilled water and placed for the periods indicated in the boiling water-bath, and subsequently diluted with the saline solutions. The vibrios in these experiments were unwashed, since we wished to reproduce the conditions of the usual agglutination reaction, in which washing is not often used.

TABLE XI.

The effect of heating at 100°C. for various periods on the potential difference in the vibrios.

Strain.	Treatment.	CONCENTRATION OF NaCl.		
		0.0093 N.	0.075 N.	0.15 N.
Rangoon Smooth.	Unheated ..	-29.9	-12.2	-6.5
	15 min. 100°C.	-36.9	-20.5	-15.8
	1 hr. „ ..	-36.0	-19.6	-14.5
	2 hrs. „ ..	-37.0	-19.5	-15.6
	4 „ „ ..	-35.1	-20.0	-15.0
2027 ..	Unheated ..	-20.8	-6.5	-2.6
	15 min. 100°C.	-33.4	-16.1	-13.2
	1 hr. „ ..	-31.9	-17.4	-13.0
	2 hrs. „ ..	-32.5	-16.9	-13.0
	4 „ „ ..	-34.0	-17.4	-13.0

TABLE XI—*concl'd.*

Strain.	Treatment.	CONCENTRATION OF NaCl.		
		0.0093 N.	0.075 N.	0.15 N.
505	Unheated ..	-26.0	-7.8	-1.6
	15 min. 100°C.	-33.8	-17.8	-13.6
	1 hr. " ..	-29.0	-14.9	-12.0
	2 hrs. " ..	-32.5	-16.9	-13.0
	4 " " ..	-31.9	-17.0	-13.2
W 880	Unheated ..	-24.2	-11.0	-3.4
	15 min. 100°C.	-36.0	-20.0	-16.1
	1 hr. " ..	-32.5	-16.0	-15.6
	2 hrs. " ..	-35.1	-19.5	-15.3
	4 " " ..	-35.1	-18.2	-14.3
1617	Unheated ..	-22.1	-6.5	-2.8
	2 hrs. 100°C.	-30.6	-16.3	-12.2

There are two main points of interest in Table XI. In the first place it is evident that heating at 100°C. is only slightly more effective in raising the *P. D.* than heating at 60°C. Whatever the underlying chemical changes may be, they occur almost to the same extent at the lower temperature. Secondly, the maximum effect of heating at 100°C. is manifested during the first 15 minutes. Continued heating up to four hours does not increase the potential, and the differences noted in the table are probably within the range of observational error in the method. In every case at the salt concentration of 0.075 N the increase in potential brings the organism well outside of the critical zone where agglutination occurs in the presence of specific sera.

The marked rise in potential at the 15-minute period may explain the observation of Gardner and Venkatraman (*loc. cit.*) that 'a few minutes' exposure to 95°C. to 100°C. is enough to remove the "H" agglutinability of a (vibrio) suspension'. In physical terms this statement means that heating the vibrios raises their surface potentials almost at once, and hence increases the repulsion between the similarly charged bacterial particles. They are accordingly less agglutinable and this change is attributed to loss of 'H' antigen. This point, and its connection with the chemical changes noted in the first part of the paper, will be considered in the 'Discussion'.

That the strain we worked with, Rangoon Smooth, actually consists of 'O' antigen after it has been heated for two hours in the boiling water-bath has been shown by serological experiments that will be reported elsewhere.

DISCUSSION.

In the data presented in this paper it is possible to distinguish two types of change in the vibrios. First are those that progress over the entire period of heating, and second those which occur within the first 15 minutes. Among the progressive changes may be noted the gradual loss of total nitrogen and amide nitrogen and the increase in humin nitrogen undergone by the residue fraction. These appear to be the result of a continuous mild hydrolysis of the vibrio. A second continuous alteration is represented by the gradual solution of A fraction in the hot water. All of these changes must disturb the reactivity of the vibrio with its homologous immune serum, both by altering the structure and by removing the highly reactive A fraction. There is also a suggestion that the structure of A which remains in the vibrio may be slightly altered.

A third progressive change occurs in the polysaccharide, which is gradually removed from the vibrio to the supernatant fluid. At the same time protein is also gradually dissolved out of the vibrio (Table V). The figures for Hausmann's analyses of the residue (Table II) and the supernatant fluid (Table VI) represent two views of the same process of progressive change in the vibrio protein. Finally, Table VIII indicates how the percentage losses of polysaccharide gradually increase with heating.

It is evident that all of the changes of this type would bring about the gradual appearance of a new structural pattern in the heated vibrios, and that the injection of this new pattern would give rise to an anti-serum differing in its range of reactivity from that against the intact organism.

The second type of change which we have found in heating the vibrios occurs in the first 15 minutes. At this period the amino-nitrogen is increased in the supernatant fluids (Table IV) and the surface potential is also increased. Further heating even to four hours does not bring about any increases comparable to those occurring at once.

All of the changes, whether gradual or rapid, are in the nature of the case connected, since they occur in and on the same vibrio. Why some occur slowly and some rapidly is a question which cannot yet be answered.

The data suggest that the destruction of 'H' antigen by heat is a complex phenomenon involving first a rapid change in the surface of the organism which is reflected in the removal of amino-nitrogen and in increased surface potential, and second by a series of slower and more progressive changes which alter the structure of the vibrio but do not further affect its surface potential. The first change destroys the 'H' antigen in the sense that the heated organisms with their higher surface charges have less or no agglutinability. The second series of changes destroys the 'H' antigen in the sense that the chemical structure is so altered that anti-serum against the heated vibrios has now a new specificity and a new range of reactivity. This latter change shows no sign of ending even after four hours' heating, and would presumably continue until the entire vibrio was in solution.

The amount of change which occurs in the destruction of 'H' antigen is shown by the following figures taken from the tables: 10 per cent of the total nitrogen, 12 per cent of the amino-nitrogen, between 10 per cent and 13 per cent of the total substance of the organisms, and from 45 per cent to 65 per cent of the polysaccharide

must be removed before the 'H' antigen disappears. It is difficult to suppose that all of this material is present in the single vibrio flagellum, and it appears probable that changes occur in the vibrio body as well.

It is also worth noting that while the amounts of material extracted from the vibrios in two hours are as shown above, all of this extract as well as the partially disintegrated vibrio has been used for injection into rabbits. In the work of Gardner and Venkatraman (*loc. cit.*), for example, the vibrios plus the extracts are injected, and the anti-sera are formed not only against the boiled vibrios, but also against any of the extracted material which has molecules large enough to cause anti-body formation.

SUMMARY.

Physical and chemical changes brought about in a vibrio strain by heat have been studied and the data correlated as far as possible with the double antigen hypothesis. The events following on heating are shown to be complex and both rapid and progressive alterations have been observed. The immediate effect of heat is to raise the surface potential and this change is correlated with the appearance of amino-nitrogen in the supernatant fluid. The long range effect of heat is to bring about a mild hydrolysis of the protein and the progressive removal of the polysaccharide from the vibrios. Both of these effects are concerned in bringing about the complex phenomenon known as destruction of the 'H' antigen.

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A STATISTICAL AND BACTERIOLOGICAL ANALYSIS OF A CHOLERA EPIDEMIC IN MANIPUR STATE, ASSAM.

BY

C. G. PANDIT, M.B., B.S., PH.D., D.P.H., D.T.M.,

E. MILFORD RICE, M.D., D.T.M. & H.,

CAPTAIN W. J. L. NEAL, M.B., B.CH., I.M.S.,

Civil Surgeon, Manipur,

AND

ASSISTANT SURGEON RAI SAHIB N. K. GHOSH, M.B., D.P.H., D.T.M.

(From the Pasteur Institute and Medical Research Institute, Shillong, Assam.)

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I. DESCRIPTION OF THE MANIPUR VALLEY.

MANIPUR STATE consists of a valley about 60 miles long and 25 miles broad, which is surrounded by hills. The mean elevation of the valley is approximately 2,500 feet above sea-level.

All the principal villages are situated on the banks of five rivers which flow through the valley from north to south, three of which leave the southern boundary of the State by a common outlet, the Manipur River, a tributary of the Chindwin River in Burma. These rivers are :—

- (a) The *Nambul*—arising in the foothills in the north-west, and emptying into the Logtak Lake.
- (b) The *Imphal* (the most important) having its source in the hills due north.
- (c) The *Iril*—a tributary of the Imphal River rising in the N. N. E.
- (d) The *Thoubal*—an important river rising in the north-east and, after draining the north-eastern part of the valley, joining the Imphal River 15 miles below the Capital.
- (e) The *Nambol*—arising in the W. N. W. and flowing into the Logtak Lake.

The courses of the Nambul, Imphal and Iril Rivers converge near Imphal where the three river beds are all within two miles of each other ; in fact the Nambul and Imphal are separated at one point by a distance of less than half a mile (*see Map*).

Imphal, the Capital, with its suburbs has a population of nearly 100,000 and is situated on the banks of the Imphal and Nambul Rivers.

The greater part of the water-supply of Imphal, which is filtered and chlorinated, is delivered to street hydrants from a distance of about 12 miles from the hills in the north-west. This supply is inadequate owing to the recent increase in the population, hence some of the inhabitants augment their requirements by utilizing the ordinary river water for domestic purposes.

II. PREVIOUS EPIDEMICS OF CHOLERA.

A widespread epidemic occurred in 1908, which took a heavy toll of life, approximately 25,000 deaths having been reported. No further details of this epidemic are available.

Another widespread epidemic of cholera occurred between May and early September 1924. According to Police reports 8,699 persons were attacked, of whom 7,327 were reported to have died. The epidemic started with isolated sporadic cases in April, and these were reported to have been introduced from the plains of Assam via the lines of communication. The general outbreak began about the 15th May. Vital statistics for Manipur State were not kept at that time (Annual Public Health Report, Assam, 1924). It was suggested then that the Police reports for 1924 did not represent the actual number of persons affected and many cases were probably missed. The Civil Surgeon in his report on this epidemic stated : ' It does not appear to have followed any special watercourse or highway of communication ', also, ' The epidemic was associated with diminished rainfall and scarcity of water '.

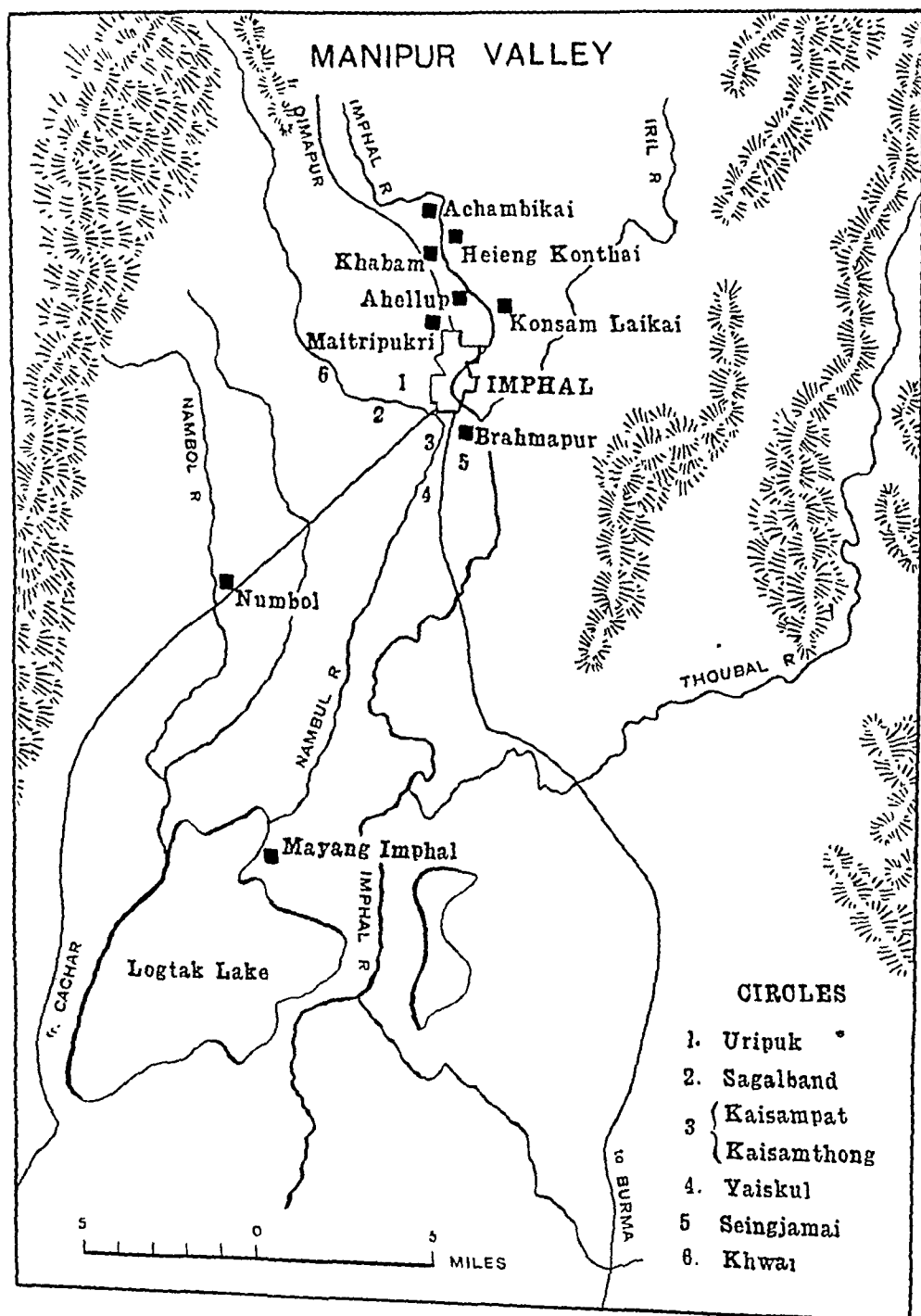
III. THE PRESENT EPIDEMIC.

The origin of the present epidemic could not be traced to any single individual case or group of cases.

Until May of that year the valley had been free from sporadic or epidemic cholera. Early in the year a large number of Manipuris had travelled to Bengal in connection with the *Ardhodaya* festival and these are reported to have returned to Manipur towards the end of April.

About November of every year there is an exodus of Manipuri labour to the plains districts of Assam for the purpose of obtaining cold weather employment. These labourers proceed principally to Lakhimpur, Sibsagar and Kamrup Districts and usually return to their homes in April. Epidemic cholera is usually present during these months in Bengal and in Kamrup. The origin of this epidemic might therefore be attributable to one or other of these factors, as sporadic cases were first discovered early in May, coinciding with the return of these travellers. Sporadic cases subsequent to May continued to be reported until July when epidemic cholera made its appearance.

MAP.



Various factors subsequently helped to spread the epidemic.

From the 2nd to 13th July, the *Ratha Yatra* festivals were held. These festivals (known as *Khechuree* festivals) entailed a succession of communal feasts where rice cooked some hours previously was consumed liberally. Previous to this, only sporadic infection was present but immediately after this festival the first crop of epidemic cases, numbering 26, was reported. A general flare up occurred the following week with 243 reported cases. These figures do not represent the total number of cases, as it was found while collecting data that some cases had not been reported, and judging by the numbers being cremated on the banks of the rivers it would be safe to assume that the Police death reports were very conservative.

Furthermore, early in the epidemic the *Shradh* ceremony with the customary religious feasting was regularly performed on the 7th and 13th day after death. This factor added further fuel to the spread of the infection and the next week (i.e., week ending the 10th August) the epidemic was at its height with 554 cases reported. At this time the feasting associated with the *Shradh* ceremony was curtailed.

Rainfall.

In 1935, until the end of July, rainfall was scanty and in consequence the river levels were low.

The rainfall for the last four years in July and August was as follows :—

Year.	July.	August.
1932 ..	12·76 inches	5·63 inches.
1933 ..	5·88 „	7·95 „
1934 ..	11·14 „	4·86 „
1935 ..	4·45 „	10·24 „

The low river levels must have been an important factor in the spread of the epidemic since the rivers were the villagers' only source of water-supply.

All the rivers, on the banks of which about 90 per cent of the population live, were ultimately infected, and it was noted that the disease was prone to spread along these watercourses.

Compared with the general sanitary condition of Indian villages in the plains of Assam, the Manipur villages are comparatively clean and tidy. Pit latrines, which are uncommon elsewhere, are to be found in all villages. However, the river banks were still used as latrines by many. During the epidemic, faecally contaminated utensils and clothing were washed in the river and the excreta of infected persons were, in many cases, thrown on to the ground and vegetable gardens in close proximity to the houses.

Between the 3rd and 16th August 8·82 inches of rain were recorded and the rivers were in high flood. This might have been one of the factors responsible for the beginning of the decline of the epidemic after that date.

IV. INOCULATION.

Mass inoculation was attempted from 8th August onwards, from when 80,000 inoculations were performed amongst a population of 290,000. It is not known how many of the total population were really at risk but, for the greater part, inoculations were given to those in the actually infected areas.*

V. TREATMENT.

Combined cholera and dysentery bacteriophage was used for some sporadic cases in the early part of, and to a great extent, during the main epidemic.

GRAPH.

Showing cases treated by different methods from 5th May to 7th September.

MANIPUR CHOLERA EPIDEMIC 1935

TREATED WITH ESSENTIAL OILS

42 Recoveries □

34 Deaths ■

Mortality 36.56 per cent

TREATED WITH BACTERIOPHAGE

226 Recoveries □

106 Deaths ■

Mortality 32.33 per cent

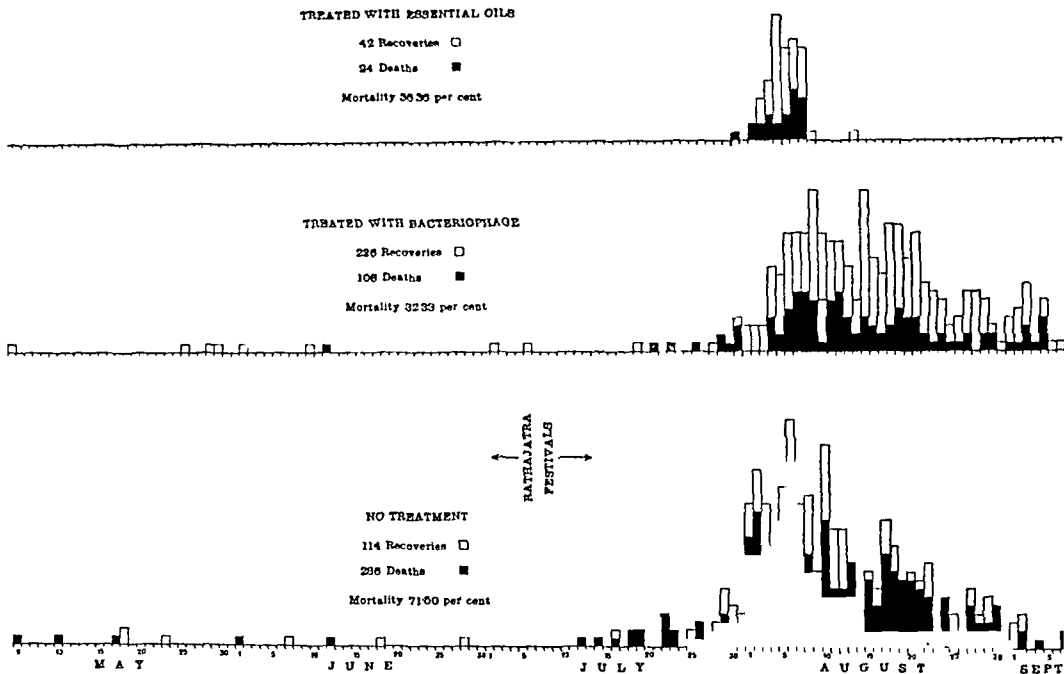
NO TREATMENT

114 Recoveries □

236 Deaths ■

Mortality 71.00 per cent

← RATHAJTRA
FESTIVALS →



In areas where bacteriophage was not readily available at first, the essential oils mixture was given. This treatment was discontinued after the 9th August when bacteriophage was made freely available everywhere (*see Graph*).

Some of the people resorted to indigenous treatment by Kavirajs.†

* The vaccine used was made at the Pasteur Institute, Shillong, had 8,000 million organisms per c.c. and was given as a single injection of one c.c.

† *Kaviraj*, locally known as *Maiba*, is a person who practises an indigenous system of medicine.

Part I. STATISTICAL.

COLLECTION OF DATA.

During August 1935, the epidemic was confined mainly to Imphal and suburbs, and to a few villages to the north and south of this area. Subsequently the epidemic extended further to the south and to groups of villages on the east and west along the foothills. Data were collected from the town and suburb areas, and from the villages to the north and south, but not from the foothills. The data are complete as regards cases, and no village was left without being fully investigated. The data of 812 cases were obtained. These investigations required three weeks of full working days. The collection of data was facilitated as the Manipur State had placed a car at the disposal of the staff and the villages were easily accessible by good roads. In addition seven doctors and four compounders, who had treated the cases in their respective villages, accompanied one of us (N. K. G.) in his visits to these villages.

The Manipur village consists of groups of 6 to 10 small houses, around a common courtyard, and four or five of such groups formed a village. Thus by visiting one courtyard, complete data could easily be collected from 6 to 10 families. These families were found to be largely inter-related, and because of the grouping of houses as described above, there was little possibility of cases being missed when each family was interrogated.

It must be emphasized that data were collected immediately the epidemic had subsided in these areas, and while the details were fresh in the minds of the villagers.

GENERAL STATISTICAL ANALYSIS.

In this epidemic we are dealing with a population which is homogeneous from the standpoint of caste and customs.

The data collected, which included those from parts affected in July, August and September, give the particulars in connection with 812 cases; of these some received no treatment and others received treatment with bacteriophage or essential oils. The general mortality irrespective of treatment was 52·22 per cent. Analysing this further we find among 400 cases falling in the 'no treatment' group there were 286 deaths, i.e., a mortality rate of 71·5 per cent. Among 279 cases which received bacteriophage treatment only, 93 died, i.e., a mortality rate of 33·33 per cent, while of 55 cases receiving bacteriophage and vaccine, 15 died, a mortality of 27·27 per cent. Those receiving essential oils treatment numbered 66, of whom 24 died, i.e., a mortality rate of 36·36 per cent. Of the balance, 12 in number, who had received no treatment but had been inoculated and of whom 9 developed symptoms on the day of inoculation, 6 died and 6 recovered.

It would appear from the above, that treatment either by bacteriophage or essential oils reduced the mortality by approximately 50 per cent as compared to the no treatment group, and that among those who had previously received vaccine and were subsequently treated with bacteriophage the mortality was still further reduced.

As had been noted in the analysis of previous epidemics (Morison, 1934). no significant effect upon the mortality by 'phage administration was noticeable when 'phage was given 48 hours after the onset of the disease. This is shown in Table I :—

TABLE I.

Day of attack when 'phage given.	Total deaths.	Total recoveries.	Percentage mortality.
1	81	188	30.1
2	12	20	37.5
3	9	5	} 45.5
4	1	7	
5	5	2	
6	0	2	
7	0	2	
TOTALS ..	108	226	32.3

It is noticed that mortality among those receiving 'phage on the 1st day was 30.1 per cent, 2nd day—37.5 per cent and in the remaining group from the 3rd to 7th day—45.5 per cent.

INOCULATION.

No attempt has been made to evaluate the effects of inoculation with cholera vaccine, but the available data in this respect, are presented.

Up to the 8th August, cholera vaccine was used only for contacts, but after that date mass inoculation was attempted. There was some reluctance shown to inoculation and only 80,000 inoculations were performed among the population of 290,000. However, this must have had some effect in checking the spread of the disease for among the 812 cases of which we present data, only 67 had been previously inoculated. These developed cholera after inoculation as follows :—

Same day	..	..	..	..	24
2nd day	..	..	..	..	15
3rd day	..	..	..	..	8
4th day	..	..	..	..	6
5th day	..	..	..	..	5
6th day	..	..	..	..	4
8th day	..	..	..	..	3
10th day	..	..	..	..	1
14th day	..	..	..	..	1

It will thus be seen that out of the 67 cases, only 5 developed the disease after the 7th day after inoculation, i.e., the time necessary for inoculation to be effective.

DETAILED STATISTICAL ANALYSIS.

We have analysed the available data from the following aspects in order to determine whether any of these factors would influence adversely or otherwise the results given in the general summary above:—

- (1) Was there any significant difference in the distribution of age and sex among cases falling in each of the three groups?
- (2) Was the reduction in mortality in the treatment groups only apparent because the fulminating cases were all included in the no treatment group, and died before any treatment could be made available to them?
- (3) Was the low mortality among the bacteriophage and essential oils groups due to a psychological factor which might operate with any form of treatment?
- (4) When all forms of treatment were available, did the population favour either one or the other?
- (5) Between 31st July and 9th August, 1935, inclusive, when essential oils and bacteriophage were both being used, were the results obtained by each method of treatment significant when compared with the untreated group of the same period?
- (6) Do the cases analysed represent the total case incidence of the area investigated and, if not, to what extent would 'missed cases', presumably untreated, influence the mortality rate of that group?

- (1) Was there any significant difference in the distribution of age and sex among cases falling in each of the three groups?

In Tables II, III and IV, the data for age and sex are given:—

TABLE II.

No treatment group—age and sex.

Age groups.	MALES.			FEMALES.			TOTALS.		
	R	D	M	R	D	M	R	D	M
1-4	10	29	74.4	8	41	83.7	18	70	79.5
5-9	17	25	59.5	13	23	63.9	30	48	61.5
10-19	12	25	67.6	13	26	66.7	25	51	67.1
20-29	6	20	76.9	17	33	66.0	23	53	69.7
30-39	5	18	78.3	4	11	73.3	9	29	76.3
40-49	3	10	76.9	2	7	77.8	5	17	77.3
50 and over	2	12	85.7	2	6	75.0	4	18	81.8
TOTALS.	55	139	71.6	59	147	71.4	114	286	71.5

R = Recoveries. D = Deaths. M = Percentage mortality.

In Table II, i.e., the no treatment group, the total mortality among 194 males was 71·6 per cent, and that among 206 females 71·4 per cent, and of the total 400 cases, 71·5 per cent. There was thus no selection in this group as regards sex. In considering the age groups, there is little evidence of any selection in any age group, with the possible exception of the group—5 to 9 years. The reduction of mortality in this group is not such as to appear of any significance.

TABLE III.

Bacteriophage group—age and sex.

Age groups.	MALES.			FEMALES.			TOTALS.		
	R	D	M	R	D	M	R	D	M
1-4 ..	19	6	24·0	12	9	42·9	31	15	32·6
5-9 ..	30	9	23·1	20	6	23·1	50	15	23·1
10-19 ..	28	9	24·3	18	11	37·9	46	20	29·9
20-29 ..	16	13	44·8	31	14	31·1	47	27	36·5
30-39 ..	13	8	38·1	15	10	40·0	28	18	39·1
40-49 ..	9	1	10·0	7	2	22·2	16	3	15·8
50 and over ..	6	7	53·8	2	3	60·0	8	10	55·6
TOTALS ..	121	53	30·5	105	55	34·4	226	108	32·3

R = Recoveries. D = Deaths. M = Percentage mortality.

From Table III, i.e., the bacteriophage group, it will be seen that there is no significant selection as regards sex. Regarding the age groups, those between the ages of 5 to 9 show a similar reduction of mortality as was noted in the no treatment group. The only other difference noted was among those of 40 to 49 years of age, with a mortality rate of 10 per cent in males and 22 per cent in females, but here the numbers are too small to be of real significance.

TABLE IV.

Essential oils—age and sex.

Age groups.	MALES.			FEMALES.			TOTALS.		
	R	D	M	R	D	M	R	D	M
1-4 ..	5	1	16.7	1	2	66.7	6	3	33.3
5-9 ..	7	1	12.5	5	3	37.5	12	4	25.0
10-19 ..	3	3	50.0	6	2	25.0	9	5	35.7
20-29 ..	1	3	75.0	6	5	45.5	7	8	53.3
30-39 ..	2	3	60.0	5	0	..	7	3	30.0
40-49 ..	0	1	..	1	0	..	1	1	..
50 and over ..	0	0	..	0	0	..	0	0	..
TOTALS ..	18	12	40.0	24	12	33.3	42	24	36.4

R = Recoveries. D = Deaths. M = Percentage mortality.

Similarly in Table IV, i.e., the essential oils group, there appears no significant selection as regards sex and the numbers in each age group are so small that no definite conclusion can be drawn from them.

The cases recorded in Tables II and III have been further distributed according to age and sex for the two groups, bacteriophage and no treatment groups. Essential oils group is not considered here, as figures under each age group are too small for such a comparison. The details are given in Table IV-A :—

TABLE IV-A.

Distribution of cases according to age and sex for the two groups, bacteriophage and no treatment.

Age groups.	MALES.		FEMALES.	
	No treatment.	Bacteriophage treatment.	No treatment.	Bacteriophage treatment.
1-4 ..	39	25	49	21
5-9 ..	42	39	36	26
10-19 ..	37	37	39	29
20-29 ..	26	29	50	45
30-39 ..	23	21	15	25
40-49 ..	13	10	9	9
50 and over ..	14	13	8	5

$n = 6$; $X^2 = 2.777$; P lies between 0.9 and 0.8. $n = 6$; $X^2 = 12.147$; P lies between 0.1 and 0.05.

The probabilities of the differences between the distributions in the case of both sexes are greater than 0.05, which is usually taken as the level of significance. Therefore, even if the age and sex of the persons attacked influenced cholera mortality, there is no reason to believe that these factors would vitiate the comparisons of mortality rates in Table V :—

TABLE V.

Mortality rates by periods.

Period.	NO TREATMENT.			BACTERIOPHAGE TREATMENT.			ESSENTIAL OILS TREATMENT.		
	R	D	M	R	D	M	R	D	M
5th May to 31st July, sporadic cases.	15	35	70.0	12	10	45.5	0	1	..
1st to 7th August ..	35	98	73.7	36	17	32.1	34	18	34.6
8th to 15th August ..	36	65	64.4	69	38	35.5	8	4	33.3
16th to 23rd August ..	15	56	78.9	67	23	25.6	..	..	..
24th to 31st August ..	8	27	77.1	26	10	27.8	..	1	..
1st to 7th September ..	5	5	50.0	16	10	38.5	..	..	..
TOTALS ..	114	286	71.5	226	108	32.3	42	24	36.4

R = Recoveries. D = Deaths. M = Percentage mortality.

In Table V the mortality rates by periods are given. In the no treatment group there is no significant reduction in mortality, period by period, throughout that portion of the epidemic examined. Similarly, in the 'phage treatment group, there is little evidence to suggest that any period had a differential influence upon the efficacy of treatment.

*This question has been further investigated by working out the X^2 for the figures of each period of cholera prevalence. The method followed was that described by Fisher ('Statistical Methods for Research Workers', 5th Edition). The results are given in Table V-A :—

TABLE V-A.

Period.	Value of X^2 ; $n = 1$	Significance ($P = 0.05$).
5th May to 31st July ..	3.9273	Significant.
1st to 7th August ..	27.8005	Significant.
8th to 15th August ..	17.2904	Significant.
16th to 23rd August ..	45.1469	Significant.
24th to 31st August ..	17.3296	Significant.
1st to 7th September ..	..	Not significant $P = 0.8127$

When the separate values of X^2 are summed, we get a quantity greater than 110, and for the corresponding sum of degrees of freedom, namely 6, this value of X^2 is definitely significant. Hence, the conclusion is that bacteriophage treatment has brought down mortality.

The essential oils treatment was only given during the period from 1st to 9th August, i.e., at the beginning of the epidemic, and is thus not comparable to the other groups from the standpoint of periods. We shall refer to this aspect again later.

- (2) Was the reduction in mortality in the treatment groups only apparent because the fulminating cases were all included in the no treatment group, and died before any treatment could be made available to them ?

*Acknowledgment.—We are indebted to Dr. K. C. K. E. Raja of the All-India Institute of Hygiene, Calcutta, for working out Tables IV-A and V-A, and comments thereon.

In this connection we accept the reasoning put forward by Morison (*loc. cit.*) and have presented our data in Table VI in the same manner :—

TABLE VI.

	No treatment.	Bacteriophage within 24 hours of onset.	Bacteriophage between 24 and 48 hours of onset.	Essential oils.
Recoveries ..	114	188	20	42
Total deaths ..	286	81	12	24
Less deaths within 24 hours of onset.	175	..	..	..
		(43 not de- ducted).	(1 not de- ducted).	(14 not de- ducted).
Nett deaths ..	111	81	12	24
Percentage mortality ..	49.3	30.1	37.5	36.4

Among the 286 deaths in the no treatment group, 175 or 59.4 per cent died within 24 hours of the onset of the disease. These were probably fulminating cases to whom any treatment may not have been available. Among 269 cases who received bacteriophage on the 1st day of illness, 81 died from 1 to 11 days later, and 43 of these died on the day of onset. The latter were presumably fulminating cases too and comparable to those who died on the 1st day in the untreated group.

If now we deduct these 175 deaths from the total deaths of the no treatment group, we would have 225 cases with 111 deaths, i.e., a mortality rate of 49.3 per cent. Even if we do not deduct the 43 deaths occurring on the 1st day in the bacteriophage group the mortality rate, when bacteriophage was administered on the 1st day, was 30.1 per cent and similarly when bacteriophage was given on the 2nd day, the mortality rate was 37.5 per cent.

Among the essential oils group of 66 cases with 24 deaths, 14 died within the first 24 hours. These latter, similar to the bacteriophage group, were presumably fulminating cases. Without deducting these from the total deaths the mortality rate is 36.4 per cent.

It will be seen that after thus abstracting no treatment deaths within 24 hours, the data presented will certainly not be weighted in favour of bacteriophage or essential oils.

- (3) Was the low mortality among the bacteriophage and essential oils groups due to a psychological factor which might operate with any form of treatment?

We have, up to this point, presented our data in accordance with the method of analysis previously adopted by Morison (*loc. cit.*). In the previous epidemics which were analysed, the no treatment group was comprised of cases which had not

received treatment in any form. In the Manipur epidemic, however, among the 400 cases which we have so far considered as 'no treatment cases', 223 had received treatment by 'Kavirajs', i.e., by those who were using an indigenous method of treatment. The reason for not excluding these cases in the beginning were two-fold : to present data comparable with that of former epidemics, when it is possible that such indigenous treatment data may not have been obtained ; and secondly, absence of precise knowledge regarding either the nature or the homogeneity of this form of treatment. It is possible that such form of treatment had some beneficial effects either medicinally or from the psychological view-point. To elicit these factors we have further analysed the so-called no treatment group in Table VII :—

TABLE VII.

Day of death.	NO TREATMENT.		' KAVIRAJI ' TREATMENT.	
	Recoveries.	Deaths.	Recoveries.	Deaths.
1	..	103	..	72
2	..	26	..	38
3	..	16	..	8
4	..	4	..	6
5	..	..	..	2
6	..	..	..	6
7	..	..	..	3
8	..	..	..	..
9	..	..	..	..
10	..	1	..	1
	27	..	87	..
TOTALS ..	27	150	87	136
Percentage mortality.	84·7		61·0	

It will be seen from this grouping that of 177 cases which received no treatment there were 150 deaths, i.e., a mortality rate of 84·7 per cent and in the Kaviraji treatment of 223 cases with 136 deaths, the mortality rate is reduced to 61·0 per cent. This reduction of nearly 24 per cent in the mortality can be attributed to three possible factors, viz. :—

1. Selection of cases.
2. Efficacy of Kaviraji treatment, and
3. A possible psychological effect of some form of treatment.

It has previously been suggested that the reduction of mortality among those treated with bacteriophage might have been due to factors 1 and 3 cited above.

In Table VII, where data for Kaviraji treatment are presented, it would appear that, while there was some sort of selection of cases receiving that treatment, they

had lived sufficiently long to receive some form of treatment and at least 72 out of 223 cases did receive treatment on the 1st day of disease. The reduction in mortality to 32·3 per cent in the bacteriophage group therefore would appear significant, because if selection, and the psychological effects of any form of treatment were the only other factors, we should have obtained a mortality rate in the bacteriophage treated group similar to that in the Kaviraji treatment group, viz., 61·0 per cent in this case.

- (4) When all forms of treatment were available did the population favour either one or the other ?

TABLE VIII.

Area.	NO TREATMENT.		KAVIRAJI TREATMENT.		BACTERIOPHAGE.		ESSENTIAL OILS.	
	R	D	R	D	R	D	R	D
<i>Town group.</i> —								
Uripuk	3	12	4	1	32	17	5	3
Sagalband	0	7	5	2	20	18	0	2
Kaisambat	1	15	4	1	36	14	0	0
Yaiskul	1	1	1	3	15	6	0	0
Seinjamai	5	11	15	5	27	15	0	0
Khwai	5	17	2	3	15	5	14	7
Brahmapur	3	18	0	0	13	7	0	0
Miscellaneous ..	0	2	2	4	2	4	0	1
TOTALS ..	18	83	33	19	169	86	19	13
Percentage mortality ..	82·2		36·5		33·7		40·6	
<i>Rural group.</i> —								
Mantripulchri ..	0	14	4	19	11	3	0	0
Khalam Ahellup ..	1	0	30	28	2	3	20	11
Haieng Konthai ..	1	20	4	31	4	4	3	0
Achambikai ..	0	0	6	26	3	0	0	0
Konsumlaikai ..	2	8	3	2	2	2	0	0
Numbol	3	9	0	2	24	5	0	0
Mayang Imphal ..	1	8	0	0	3	1	0	0
Miscellaneous ..	1	8	7	9	8	4	0	0
TOTALS ..	9	67	54	117	57	22	23	11
Percentage mortality ..	88·2		68·4		27·8		32·4	
GRAND TOTALS ..	27	150	87	136	226	108	42	24
GRAND PERCENTAGE MORTALITY.	84·7		61·0		32·3		36·4	

R = Recoveries.

D = Deaths.

In Table VIII, we have presented the data of cases in two groups: (a) those cases occurring in Imphal Town or within a radius of three miles and (b) those cases occurring at a further distance from the town.

These cases are distributed in Table IX according to the respective treatment which they received:—

TABLE IX.

Treatment.	TOWN GROUP (440 CASES).		RURAL GROUP (360 CASES).	
	Number.	Percentage.	Number.	Percentage.
No treatment ..	101	23.0	76	21.1
Kaviraji ..	52	11.8	171	47.5
Bacteriophage ..	255	58.0	79	21.9
Essential oils ..	32	7.2	34	9.5

It will be seen from Table IX that in the town group, where presumably all three forms of treatment were easily available, that the population preferred bacteriophage treatment to Kaviraji treatment, 58 per cent of the total cases having received the former treatment, compared to 11.8 per cent of the latter.

With respect to essential oils they were only used between the 30th July and 9th August principally in the Khwai and Khalam Ahellup Circles before bacteriophage was freely available..

In the rural group 47.5 per cent of the total cases received Kaviraji treatment and only 21.9 per cent received bacteriophage. • It would appear that in the rural areas bacteriophage was not so freely available, and so the population resorted to the only form of treatment which they could get, i.e., the Kaviraji treatment. This statement is borne out by the fact that in Numbol, which is on the Cachar road and readily accessible to the town, 29 cases out of total 43 cases received bacteriophage. Similarly, in Mantripulchri, we found that Kaviraji treatment was used extensively from the 1st to 15th August, when it was practically entirely replaced by the use of bacteriophage.

It would appear, therefore, that the people had confidence in the treatment made available by Government agencies, and took that treatment readily. If there was any preference for treatment it was for the bacteriophage as soon as it was made available.

In Table VIII, when comparing the town and rural areas according to the no treatment, bacteriophage and essential oils groups it will be seen that the respective

mortalities under these headings in both areas were significantly close to the mean mortality rate for each respective group. In the Kaviraji treatment, however, the mortality rate in the rural area (68·4 per cent) is considerably higher than that of the town group, viz., 36·5 per cent. This reduction in mortality in the latter is indeed comparable to that obtained either by 'phage or essential oils. We are unable to explain this difference in the Kaviraji treatment mortality rates in the two areas in the absence of definite knowledge of the general nature of that treatment*.

- (5) Between 31st July and 9th August, 1935, inclusive, when essential oils and bacteriophage were both being used, were the results obtained by each method of treatment significant when compared with the untreated group of the same period ?

TABLE X.

Analysis of cases occurring between 31st July and 9th August.

	No treatment.	Kaviraji.	Bacteriophage.	Essential oils.
Recoveries ..	9	37	56	42
Deaths ..	61	58	33	24
TOTALS ..	70	95	89	66
Percentage mortality.	87·1	61·1	36·4	37·1

It will be seen from Table X, that the mortality rates in the bacteriophage and essential oils groups would appear to be significantly lower than those of the Kaviraji or 'no treatment' groups. It will be seen also that the total cases in all the four groups are comparable in numbers—a significant point in this table.

- (6) Do the cases analysed represent the total case incidence in the area investigated and, if not, to what extent would 'missed cases', presumably untreated, influence the mortality rate of that group ?

The above data were collected by one of us (N. K. G.), who visited every affected house in the area investigated. Other houses were also visited, to obtain data

* It may be interesting to note that in the course of the investigation, it was found that a Kaviraj was treating cases with the clear supernatant fluid obtained from a stool of a cholera case, and another Kaviraj had used this treatment during an epidemic in 1924 for the treatment of his son.

should a mild case not have been reported, but every inmate was not questioned. Early cases in May and June, not previously reported, as well as some unreported cases occurring during the epidemic period, were thus found by this procedure. However, it is possible that some milder cases might yet have remained undetected. If so, these cases were obviously untreated cases, and would thus fall into the 'no treatment' group. In recognizing this factor, we have attempted to ascertain to what extent these undetected cases may have been present, in order to vitiate significantly the mortality rate shown in the 'no treatment' group.

As shown in Tables VII and VIII there were a total of 177 cases who did not receive treatment of any description. In order to reduce the mortality in this group, i.e., 84.7 per cent to 61.0 per cent, observed with the Kaviraji treatment or 32.3 per cent with bacteriophage, it would have been necessary for us to have missed 68 cases in the first instance and 288 cases in the latter.

In view of the detection of previously unreported cases, it is not likely that 68 cases could have been missed and it seems almost improbable that 288 cases, i.e., 36 per cent of the total cases recorded, could possibly have remained undetected.

INFECTIVITY.

We have used Morison's formula as a measure of infectivity, i.e., the index C , the ratio of $y\sqrt{\frac{a+b}{2}}$ multiplied by 1,000, where a =primary case, b =the remaining persons in the same house, and y =the secondary cases in the same house.

We show in Table XI, the value of C for 6 group headings:—

TABLE XI.

	Combined no treat- ment and Kaviraji treatment groups.		No treat- ment.		Kaviraji treatment.		Bacterio- phage within 48 hours.		Bacterio- phage after 48 hours.		Essential oils.	
	1	2	1	2	1	2	1	2	1	2	1	2
Primary cases in a house.												
Total popula- tion.	1,509	131	709	51	800	80	1,025	55	202	0	278	20
Primary cases (a).	275	45	128	16	147	29	182	19	37	0	47	7
Population at risk (b).	1,234	86	581	35	653	51	843	36	165	0	231	13
Secondary cases (y).	191	8	50	6	51	2	38	1	22	0	12	5
Value of C ..	133.9	122.1	141.0	235.3	127.5	50.0	74.1	36.4	217.8	0	80.5	500

When there was one primary case in a house the infectivity rates were as follows :—

No treatment group	..	..	..	141·0
Kaviraji treatment	..	..	..	127·5
Bacteriophage within 48 hours		..	..	74·1
Bacteriophage after 48 hours		..	..	217·8
Essential oils	..	..	..	80·5

It would appear that bacteriophage and essential oils had reduced the infectivity ratio to some extent. Bacteriophage given after 48 hours shows a higher infectivity ratio than do either the no treatment or the Kaviraji treatment groups. These cases, as they lived for 48 hours before receiving bacteriophage, were as infective as the no treatment or Kaviraji treatment groups cases or more so, since the majority of cases in the two latter groups had died within 24 hours.

On the assumption that cases surviving after 24 hours are more likely to give rise to secondary cases than those who have died within this time, we have deducted such deaths from each group, and have calculated the infectivity rates of those surviving after 24 hours according to value *C* by the same formula. The altered infectivity rates were as follows :—

No treatment	..	..	..	316·5
Kaviraji treatment	..	..	..	140·1
Bacteriophage within 48 hours		..	..	77·4
Bacteriophage after 48 hours		..	..	217·8
Essential oils	..	..	..	90·6

From these figures it would appear that the infectivity ratio is reduced with any form of treatment.

While it is possible that bacteriophage may alter the infectivity of the vibrio by its biological action, we are unable to explain the reduction in the value of *C* in the essential oils and Kaviraji treatment groups unless we assume that those who resort to any form of treatment also take hygienic precautions against infection in the presence of a primary case. Indeed field experience has shown that in certain houses more intelligence is shown than in others regarding hygienic measures.

Part II.

BACTERIOLOGICAL FINDINGS.

* Immediately on receipt of the report of the epidemic, a field unit in charge of a bacteriologist was sent to Imphal to investigate the epidemic bacteriologically. The unit worked at Imphal from the 11th August to 1st September, during which period specimens of stools from 70 cases from different areas were examined and from these 37 specimens yielded vibrios which were identified as cholera vibrios by the usual laboratory tests. Some of the specimens were obtained from cases who had received no treatment, some within 24 hours, after bacteriophage was administered to them, and from some to whom bacteriophage was given at least 48 hours before the specimens were taken.

The specimens were examined within four hours of collection. A flake of mucus was selected, washed in either broth or saline, and plated direct on to a bile-salt agar plate. When flakes were not obtainable, a loopful of material was plated after preliminary enrichment in 1 per cent peptone water.

In the study of the vibrios the following sugars were used : Lactose, glucose, dulcitol, mannitol, saccharose and maltose.

For agglutination tests, the following high titre sera were used :—

1. Serum *vs.* *Vibrio* 653 Titre 1-5,000. 'OH'.
2. Serum *vs.* *Vibrio* Inaba Titre 1-1,000. 'O' only.
3. Serum *vs.* *Vibrio* Ogawa Titre 1-1,000. 'O' only.

Sera Nos. 2 and 3 were obtained from the Director, Central Research Institute, Kasauli.

The general findings are given in Tables XII, XIII and XIV :—

In Table XII particulars of eight vibrios from cases receiving no treatment are listed. All gave typical sugar reactions, and were smooth to Millon's test. The first five gave typical agglutination reactions with Inaba and 653 (Shillong) sera and differential agglutinations with Ogawa serum (Gardner and Venkatraman, 1935). These vibrios are therefore typical cholera vibrios. The remaining three strains gave an indication of a different antigenic structure, especially when tested with Inaba serum. SM 47 also shows great reduction in titre with serum 653.

In Table XIII, particulars of 23 strains isolated from cases within 24 hours of their receiving bacteriophage are given. All gave typical sugar reactions, though one fermented dulcitol in addition and all were smooth to Millon's test. Their agglutination reactions are typical with the three sera used ; the slight variations which are noticed, require no comment.

* The strains collected were investigated at the Pasteur Institute, Shillong, by Doctors A. C. Vardon, I.M.D., C.P.H., N. M. Maitra, B.Sc., M.B., and N. C. Roy, L.M.P., L.T.M.

TABLE XII.

Vibrio isolated from 410 treatment cases.

Strain No.	Date of onset.	Date specimen collected.	Patient recovered or died.	Sugar reactions.	Indol test.	Cholera red test.	Millon's test—Smooth or Rough.	Hemolysis (sheep cells).	AGGLUTINATION TESTS—SER.		
									Inaba ('O').	Ogawa ('O').	653 ('OH').
SM 12 ..	11-8-35	11-8-35	Died	Typical	++	—	S	Non-haemolytic	1,000	250	5,000
SM 29 ..	16-8-35	16-8-35	"	"	++	Tr.	S	"	1,000	250	5,000
SM 30 ..	16-8-35	17-8-35	"	"	++	Tr.	S	"	1,000	250	5,000
SM 32 ..	16-8-35	19-8-35	"	"	++	+	S	"	1,000	250	5,000
SM 37 ..	19-8-35	20-8-35	Recovered	"	++	—	S	"	1,000	500	2,500
SM 47 ..	17-8-35	23-8-35	"	"	+	—	S	"	Tr. in all dil. to 1,000.	125	Tr. in all dil. to 125.
SM 57 ..	28-8-35	29-8-35	Died	"	++	+	S	"	Tr. in all dil. to 2,500.	125	2,500
SM 65 ..	1-9-35	3-9-35	"	"	++	+	S	"	Tr. in all dil. to 2,500.	125	2,500

TABLE XIII.

Vibrios isolated from cases receiving bacteriophage.

(Specimens obtained within 24 hours of receiving bacteriophage.)

Strain No.	Date of onset.	Date specimen collected.	Patient recovered or died.	Sugar reactions.	Indol test.	Cholera red test.	Millon's test—Smooth or Rough.	Hæmolysis (sheep cells).	AGGLUTINATION TESTS—SERA.		
									Inaba ('O').	Ogawa ('O').	653 ('OH').
SM 2 ..	10-8-35	12-8-35	Recovered	Typical	++	++	S	Non-hemolytic	1,000	250	2,500
SM 3 ..	10-8-35	12-8-35	"	"	++	++	S	"	1,000	250	2,500
SM 4 ..	10-8-35	12-8-35	"	"	++	++	S	"	1,000	125	1,000
SM 5 ..	10-8-35	12-8-35	"	"	++	++	S	"	1,000	125	2,500
SM 6 ..	12-8-35	14-8-35	"	"	++	++	S	"	1,000	250	2,500
SM 16 ..	12-8-35	14-8-35	"	"	++	++	S	"	1,000	125	2,500
SM 17 ..	12-8-35	14-8-35	Died	"	++	++	S	"	1,000	125	2,500
SM 18 ..	12-8-35	14-8-35	"	"	++	++	S	"	1,000	125	2,500
SM 20 ..	13-8-35	15-8-35	"	"	++	++	S	"	1,000	500	5,000
SM 21 ..	14-8-35	15-8-35	Recovered	"	++	Tr.	S	"	1,000	250	5,000
SM 23 ..	14-8-35	16-8-35	Died	"	++	Tr.	S	"	1,000	250	5,000
SM 24 ..	15-8-35	16-8-35	"	"	++	Tr.	S	"	2,500	125	5,000
SM 25 ..	15-8-35	16-8-35	Recovered	"	++	Tr.	S	"	1,000	250	5,000
SM 26 ..	15-8-35	16-8-35	"	"	++	Tr.	S	"	2,500	250	5,000
SM 27 ..	18-8-35	20-8-35	"	"	++	Tr.	S	"	1,000	250	5,000
SM 28 ..	14-8-35	20-8-35	"	"	++	Tr.	S	"	1,000	125	5,000
SM 31 ..	17-8-35	20-8-35	"	"	++	+	S	"	1,000	250	2,500
SM 35 ..	18-8-35	20-8-35	"	"	++	-	S	"	1,000	500	1,000
SM 36 ..	19-8-35	20-8-35	"	Typical but dulcitate also fermented.	++	+	S	"	1,000	250	2,500
SM 38 ..	19-8-35	20-8-35	Died	Typical	++	+	S	"	1,000	250	1,000
SM 42 ..	21-8-35	22-8-35	Recovered	"	++	Tr.	S	"	1,000	250	5,000
SM 43 ..	21-8-35	22-8-35	"	"	++	Tr.	S	"	2,500	250	2,500
SM 44 ..	21-8-35	22-8-35	Died	"	++	+	S	"	500	125	2,500
SM 46 ..	22-8-35	23-8-35	Recovered	"	++	+	S	"	500	50	2,500

TABLE XIV.

Vibrios isolated from cases receiving bacteriophage.

(Specimens obtained after 48 hours of receiving bacteriophage.)

Strain No.	Date of onset.	Date specimen collected.	Patient recovered or died.	Sugar reactions.	Indol test.	Cholera red test.	Millon's test—Smooth or Rough.	Hemolysis (sheep cells).	AGGLUTINATION TESTS—SERA.		
									Inaba ('O').	Ogawa ('O').	653 ('OH').
SM 7 ..	9-8-35	13-8-35	Recovered	Typical	++	—	S	Non-hemolytic	1,000	125	2,500
SM 13 ..	11-8-35	14-8-35	Died	"	++	—	S	"	1,000	125	2,500
SM 33 ..	13-8-35	19-8-35	Recovered	"	++	—	S	"	Tr. in all dil. to 500.	Tr. in all dil. to 125.	Tr. in all dil. to 1,000.
SM 39 ..	15-8-35	20-9-35	"	Typical but dulcitate also fermented.	++	—	S	"	500	Tr. in all dil. to 125.	5,000
SM 40 ..	16-8-35	21-8-35	"	Typical	+	Tr.	S	"	2,500	125	500 and tr. up to 5,000
SM 45 ..	18-8-35	21-8-35	"	"	++	+	S	"	250	Tr. in all dil. to 125.	250 and tr. up to 5,000

In Table XIV particulars of six strains are given. The stools were collected in these cases at least 48 hours after they had been treated with bacteriophage. These are listed separately to ascertain whether their antigenic structure had been altered by the administration of bacteriophage. All gave typical sugar reactions except one which also fermented dulcitol. All were smooth to Millon's test. The agglutination reactions of the first two are typical. The others show variations as were noticed in Table XII, but in this case the variations were also noticeable with the Ogawa serum.

A perusal of Tables XII, XIII and XIV would suggest, that atypical agglutination reactions were obtained in all groups in vibrios isolated at the end of the epidemic. It was also found that between 23rd August and 3rd September, although 23 specimens were examined only two gave positive results, i.e., SM 57 and SM 65.

We have also investigated the lysability or resistance of strains to 'phage types, and the action of different brews of therapeutic 'phage issued for use in the field. The therapeutic brews used for these tests had the following composition :—

Brew No.	Types present.	Strength.
419	A B C D G K	1×10^{12}
422	A B C D E F G H J K	1×10^{12}
423	A B C D E F H J K	1×10^9
424	A B C D E F G H J	1×10^{12}
430	A B C D F G H J	1×10^{11}
431	A B C E G H J	1×10^{10}

The readings regarding lysability of cultures by these brews were taken at the end of four hours. At the end of 24 hours only minor variations were noted. The results are given in Tables XV, XVI and XVII.

TABLE XV.

Action of cholera bacteriophages on vibrios listed in Table XII.

(Readings taken after four hours.)

Strain No.	ACTION OF INDIVIDUAL TYPES OF CHOLERA PHAGES.		LYSABILITY TO THERAPEUTIC PHAGE BREWS.					
	Lysed by types.	Resistant to types.	419	422	423	424	430	431
SM 12 ..	BCDEFHJ	AGKL	+++	++	++±	++±	+++	+++
SM 29 ..	BCDEFHJ	AGKL	+++	++	+++	+++	+++	+++
SM 30 ..	BCDEFHJ	AGKL	++	++	++	+++	+++	+++
SM 32 ..	BCDEFHJ	AGKL	++±	++	+++	+++	+++	+++
SM 37 ..	BCDEFHJKL	AG	+++	++	++	++±	+++	+++
SM 47 ..	Nil	All types	—	—	—	—	—	—
SM 57 ..	BCDFHJ	AEGKL	*	*	*	++±	*	*
SM 65 ..	BCDEFHJ	AGKL	*	*	*	±	*	*

* Not tested.

In this, and subsequent tables, the results of lysability tests are shown thus:—

+++ = complete lysis.
 ++±
 ++
 +±
 ±
 +
 — = no lysis.

} = partial lysis and gradations.

TABLE XVI.

Action of cholera bacteriophages on vibrios listed in Table XIII.

Strain No.	ACTION OF INDIVIDUAL TYPES OF CHOLERA 'PHAGES.		LYSABILITY TO THERAPEUTIC 'PHAGE BREWS.					
	Lysed by types.	Resistant types.	419	422	423	424	430	431
SM 2 ..	C D E F G H J K L	A B	++	+++	+++	+++	+++	+++
SM 3 ..	B C D E F G H J	A K L	+++	++±	++	+++	+++	+++
SM 4 ..	B C D E F H J	A G K L	++±	++	++	+++	+++	+++
SM 5 ..	B C D E F H J	A G K L	+++	++	++	++±	+++	+++
SM 6 ..	B C D E F G H J K L	A	++	+++	+++	+++	+++	+++
SM 15 ..	B C D E F H J	A G K L	+++	++±	++	+++	+++	+++
SM 17 ..	B C D E F H J	A G K L	++±	++	±	±	+++	+++
SM 18 ..	B C D F G H J	A E K L	+++	++	++	++	+++	+++
SM 20 ..	B C D E F H J	A G K L	++±	++	±	++±	+++	+++
SM 21 ..	B C D E F H J	A G K L	--	++	++	+++	+++	+++
SM 23 ..	B C D E F H J	A G K L	+++	+++	+++	+++	+++	+++
SM 24 ..	B C D F G H J	A E K L	+++	+++	+++	+++	+++	+++
SM 25 ..	B C D F G H J	A E K L	+++	+++	+++	+++	+++	+++
SM 26 ..	B C D F G H J	A E K L	+++	+++	+++	+++	+++	+++
SM 27 ..	B C D E F H J	A G K L	+++	++	+++	+++	+++	+++
SM 28 ..	B C D E F H J	A G K L	+++	+++	++±	+++	+++	+++
SM 31 ..	B C D E F H J	A G K L	+++	+++	+++	+++	+++	+++
SM 35 ..	B C D E F H J	A G K L	+±	±	±	+	+++	+++
SM 36 ..	B C D E F H J	A G K L	+	+	+	±	±	±
SM 38 ..	B C D E F G H J	A K L	+++	++	+++	++	+++	+++
SM 42 ..	B C D E F G H J	A K L	++±	++±	+++	+++	+++	+++
SM 43 ..	B C D E F G H J	A K L	+++	+++	+++	+++	+++	+++
SM 44 ..	B C D E F H	A G J K L	±	++	+++	+++	+++	+++
SM 46 ..	B C D E F H	A G J K L	+++	±	±	±	+++	+++

TABLE XVII.

Action of cholera bacteriophages on vibrios listed in Table XIV.

Strain No.	ACTION OF INDIVIDUAL TYPES OF CHOLERA 'PHAGES.		LYSABILITY TO THERAPEUTIC 'PHAGE BREWS.					
	Lysed by types.	Resistant to types.	419	422	423	424	430	431
SM 7 ..	B C D E F H J	A G K L	++±	++	++±	++±	+++	+++
SM 13 ..	B C D E F G H J	A K L	+++	++	+++	++±	+++	+++
SM 33 ..	Nil	All types	—	—	—	—	—	—
SM 39 ..	B C D E F H J K L	A G	++	+++	+++	+++	+++	+++
SM 40 ..	B C D E F G H J	A K L	+++	++	++±	++±	+++	+++
SM 45 ..	B C D E F G H J K L	A	++±	+++	+++	+++	+++	+++

It will be seen in these tables that two strains only, viz., SM 33, SM 47, were resistant to all types of cholera bacteriophages. These strains, as might be expected, were resistant to therapeutic 'phage brews. Their agglutination reactions were also atypical, but other characters such as fermentation of sugars, etc., were typical of a cholera vibrio. Strains SM 6 and SM 45 were resistant to type 'A' 'phage only, though only the former showed typical agglutination. In the action of therapeutic 'phage on these strains, a similar difference was noted, in that brew No. 419 did not give complete lysis in four hours. Twenty strains were resistant to A, G, K and L types. The therapeutic 'phage brews show variations in lysability though their agglutination reactions of these strains were more or less typical.

Summarizing these results we find that in all the three groups, the biochemical characters of the vibrios isolated simulate one another. Those vibrios isolated in the early part of the epidemic in all instances give true agglutination reactions. Towards the end of the epidemic variations in agglutinability are noticeable.

As regards resistance to individual 'phage types, the three groups do not differ materially. There was no correlation between 'phage resistance and agglutinability. With two exceptions the therapeutic 'phage lysed all the vibrios isolated. In studying the action of different brews on the individual vibrios, variations in lysability were noticed. This is important, for as the brews may differ in their action because of the absence in them of one or more 'phage types, varying results in the use of therapeutic 'phage in the field are possible.

SUMMARY.

- (1) A cholera epidemic in the Manipur Valley of Assam is described.
- (2) The general statistical analysis shows that the mortality in 812 cases, regardless of treatment, was 52·2 per cent, that among 400 cases receiving no treatment 71·5 per cent, among 334 cases treated with bacteriophage, 32·3 per cent, and among the 66 treated with essential oils 36·4 per cent. Of the 812 cases, 67 had been inoculated. The majority of cases are thus found to be amongst the uninoculated.
- (3) The detailed statistical analysis is given to evaluate the question of possible selection in the treated groups; any psychological factor operating amongst treated cases; whether the population favoured one or other form of treatment; and whether the cases recorded represented the total incidence of the disease in the area, and if any cases had been missed to what extent they influenced the statistical analysis.
- (4) In previous epidemics, we had no knowledge as to whether indigenous form of treatment was used during an epidemic. In this epidemic such treatment was given by Kavirajs. The influence of this factor is discussed. This factor has also facilitated the analysis noted above.
- (5) The infectivity, as evaluated by Morison's formula, is given. The ratio was lower both in the bacteriophage and essential oils groups. The influence of any form of treatment on infectivity is discussed.
- (6) The epidemic has been investigated bacteriologically and was found to be due to a typical agglutinating vibrio. Details are given of cholera vibrios obtained from those who received no treatment, and of others isolated at different periods after the cases had received bacteriophage treatment.

ACKNOWLEDGMENTS.

Our thanks are due to the Maharaja of Manipur for placing the resources of the state at our disposal, Colonel C. E. Palmer, I.M.S., Inspector-General of Civil Hospitals, Assam, and Lieut.-Colonel T. D. Murison, I.M.S., Director of Public Health, Assam, for their assistance and to Dr. R. B. Lal and Dr. K. C. K. E. Raja of the All-India Institute of Hygiene, Calcutta, for their valuable suggestions in the preparation of this paper. We are also indebted to Mr. A. B. Smart for the preparation of the Map.

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AN EPIDEMIC OF CHOLERA IN MONDAIR VILLAGE (HABIGANJ SUBDIVISION, ASSAM).

BY

C. G. PANDIT, M.B., B.S., PH.D., D.P.H., D.T.M.,

AND

E. MILFORD RICE, M.D., D.T.M. & H.

(From the Pasteur Institute & Medical Research Institute, Shillong, Assam.)

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THE Habiganj subdivision was selected in 1932 as one of two areas in Assam, in which to determine the effects of bacteriophage in the control of cholera epidemics. The principles underlying this form of control have already been described by Morison *et al.* (1933). By the distribution of bacteriophage to each village, early treatment of cases of cholera is facilitated. An account is given here of an outbreak of cholera in a village in this area.

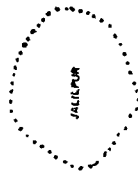
Mondair is a small village situated on the southern bank of the Ratna, a tributary of the Barak River and at a distance of about 10 miles from Habiganj Town. The village runs east to west for about five furlongs along the river bank. It consists of a series of huts built on small artificial mounds, about 12 in number, situated in most cases very close to each other. The total population is 751, Mohammedans predominating. The river is the only source of water-supply, excepting one tank adjacent to, and used by, one small group of huts only (*see Map*).

THE ORIGIN OF THE OUTBREAK.

It was ascertained that during the night of 2nd April, a Hindu lady, a pilgrim returning from a festival in Lakhai (on the banks of the Dhalisiri River bordering on Mymensingh District), passed through this village and, crossing the river at the Ferry Ghat, proceeded towards Baniachung. She was suffering from vomiting and diarrhoea, and was so ill that she had to be carried in a 'Sowari'. At the Ferry Ghat she had to wait for about three hours before arrangements could be made for her transport. It is suspected that this case was the cause of the importation of cholera into Mondair. At this Ferry Ghat on the following day (3rd April), a woman of Mondair waited all day from the early hours of the morning, to receive her relatives who were due to arrive from Baniachung. During the night this woman developed cholera and died. This was the first case in the village and this case gave rise to an epidemic resulting in 65 attacks and 33 deaths. The last case was reported on

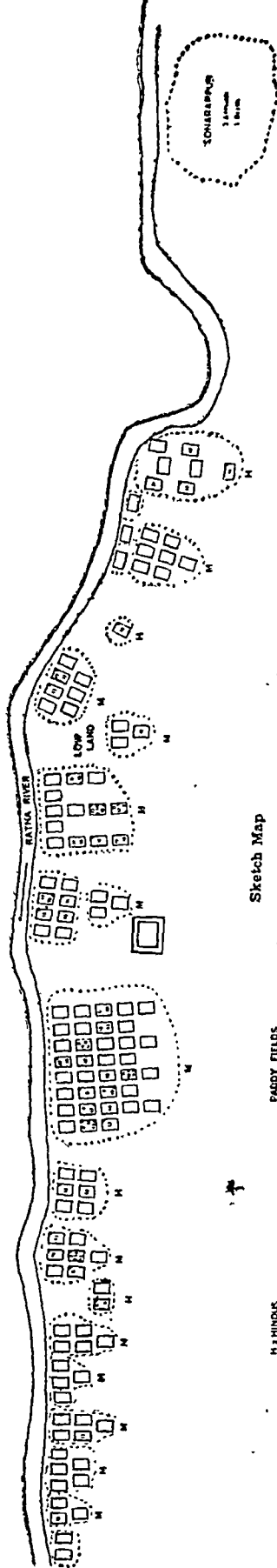
Cholera Epidemic, April 1935

MAP



PADDY FIELDS

PADDY FIELDS



Sketch Map

VILLAGE MONDAIR

THANA BANITACHONG
SUB-DIVISION HABIGANJ
DISTRICT SYLHET

PADDY FIELDS

PADDY FIELDS

H = HINDUS

M = MUHAMMADANS

Colors of Red Line in Report

Red	Blue	Black

22nd April, 1935. The dates on which the cases occurred in different houses, and the total number of cases and deaths in each group of huts are recorded in the Map. It was ascertained that during the epidemic, i.e., between the 3rd and 22nd April, there had occurred a large number of cases of diarrhoea which had received bacteriophage but which were not recorded as cholera. Some of these appear to have been cases of cholera. The evidence in this connection will be discussed under 'Comments' at the end of this paper.

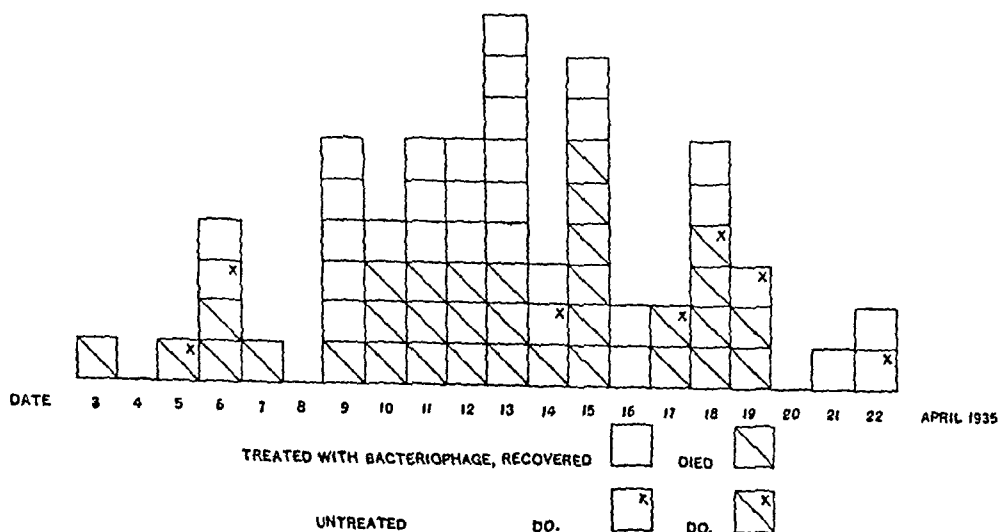
Although some early cases may have been infected through the medium of the river water, it does not appear that the bulk of the epidemic was due to the contamination of river water which was the only source of supply. While a few cases occurred in houses situated down the stream, the epidemic spread rapidly eastwards and reached Sonarampur village, about half a mile up stream from Mondair by the 14th April. Western portions of Mondair itself, and Jalalpur village, situated on the immediate opposite bank of the river, and a few villages down stream within two miles of Mondair, escaped entirely. The bulk of the epidemic spread was, therefore, by human contact, or through flies, which were particularly prevalent at the time.

RESULTS OF TREATMENT WITH BACTERIOPHAGE.

At the time of the outbreak there was a sufficient supply of bacteriophage available with the village stockists for the treatment of earlier cases. In addition,

GRAPH

Cholera epidemic, village Mondair, Habiganj.



bacteriophage was distributed to each household on the 16th April, so that it might be available for immediate use in any household. The first case (3rd April) had

bacteriophage about six hours after the onset of symptoms. This patient died four days later.

In all there were 65 attacks with 33 deaths (*see Graph*). Other cases, as mentioned above, are not included in this analysis. Of the 65 cases, seven cases received no treatment. It appears they were prevented from taking any medicine by a Pir who had visited the village. Fifty-eight cases were, therefore, treated with bacteriophage. Of these, two cases besides having bacteriophage, had some mixtures and powders as well, given by a private practitioner of Baniachung, and one case took bacteriophage in hot water. Omitting these three cases the results obtained in the 55 cases treated with bacteriophage only were analysed :—

TABLE I.

	Total cases.	Deaths.	Percentage mortality.
Treatment with bacteriophage.	55	27	49
No treatment ..	7	3	..

Some cases had bacteriophage immediately and others a few hours after the onset of symptoms. That there was no marked difference in the mortality rates in these sub-groups is shown below :—

TABLE II.

Administration of bacteriophage.	Cases.	Deaths.	Percentage mortality.
Immediately ..	39	18	46
Within 6 hours ..	14	7	50
12 to 18 hours ..	2	2	100

The time factor being of importance in bacteriophage as well as any other form of treatment, when dealing with a virulent infection it is necessary to ascertain whether bacteriophage had sufficient time to act and be of some therapeutic value.

To show this factor the 27 deaths in the 'phage group are further analysed in Table III :—

TABLE III.

Day of death after bacteriophage administration.	Number of deaths.	Remarks.
1st day ..	8	5 had 'phage immediately.
2nd day ..	6	4 had 'phage immediately.
3rd day ..	5	3 had 'phage immediately.
4th day ..	4	2 had 'phage immediately.
5th day ..	1	} had 'phage immediately.
6th day ..	1	
7th day ..	1	
9th day ..	1	

From the above table it appears that of 27 cases that died, 19 had lived for 24 hours or more after the administration of bacteriophage. In spite of early administration of bacteriophage the mortality rate in this epidemic was high.

The question whether bacteriophage had reduced the infectivity of the vibrio was then investigated. In all there were 35 primary cases, of which 15 gave rise to 22 secondary cases. Full details of these 15 primary cases and their secondaries are given in Table IV :—

TABLE IV.

Primary case.	Recovered or died.	Bacteriophage when administered.	Number of secondary cases.	Number of days after the primary case.	Population in the house.
1	Died 1st day ..	Immediately	1	3 days	5
1	Died 4th day	6 hours after onset	2	5 days and 7 days	6
1	Died 7th day	Immediately	2	3 days and 4 days	10
1	Recovered ..	Immediately	1	2 days	6
1	Recovered ..	Immediately	2	2 days and 3 days	8
1	Died 3rd day	Within 5 hours	1	4 days	7
1	Recovered ..	Immediately	3	3 days 3 days 7 days	8
1	Recovered ..	6 hours	1	7 days	10
1	Recovered ..	Immediately	1	5 days	5
1	Recovered ..	Immediately	1	8 days	8
1	Recovered ..	Immediately	1	3 days	6
1	Recovered ..	5 hours	1	5 days	5
1	Recovered ..	5 hours	2	8 days and 10 days	7
1	Died 2nd day	Immediately	1	3 days	6
1	Died 6th day	Immediately	2	1st day, 5 days	7

It will be seen that nine cases out of these 15 primary cases had recovered. Twenty-one secondary cases developed from two to eight days and one in 10 days after the primary case. All the primary cases had received bacteriophage within five hours from the onset of symptoms. It is interesting to note that of seven cases which did not receive any treatment, three were primary cases which did not give rise to any secondaries. The figures in the no treatment group are, however, too small for any significant comparison to be made.

It appears that bacteriophage had had little influence in this epidemic in reducing the infectivity of the vibrio to actual contacts.

COMMENTS.

This is the second village epidemic in which the bacteriophage had apparently failed to reduce the case mortality appreciably. The first outbreak was at Radhala in the Nowgong District in 1934 with 22 attacks and 16 deaths.

As is well known, the case mortality varies in different epidemics, and, in the absence of a significant number of cases receiving no treatment with which to judge the virulence of the epidemic, it would be difficult to determine whether bacteriophage had really failed or not. We have in this connection three instances of epidemics of cholera in this province, viz., the Sibsagar, Darrang and Cachar epidemics which on investigation showed varying degrees of virulence (Morison *et al.*, 1933, 1934). The mortality rates in these epidemics are shown in Table V:—

TABLE V.

Epidemics.	CASES RECEIVING NO TREATMENT.		BACTERIOPHAGE TREATMENT WITHIN 48 HOURS OF ONSET.	
	Cases.	Percentage mortality.	Cases.	Percentage mortality.
Sibsagar ..	399	48.1	124	23.4
Darrang ..	138	83.3	67	44.5
Cachar ..	958	70.0	641	21.2

If then bacteriophage had been used for the treatment of *all cases* in the Darrang epidemic (as was done in the Mondair outbreak) and the total case mortality had been reduced to 44 per cent as in the bacteriophage cases, and these figures were then compared with the 48.1 per cent mortality rate in the untreated group of the Sibsagar epidemic, the beneficial results of the bacteriophage treatment in Darrang would not have been apparent. In the Darrang outbreak we must have been dealing with a vibrio of different virulence than that of the Sibsagar epidemic. The mortality rate in the Mondair outbreak would suggest that the virulence of the responsible vibrio was comparable to that of the Darrang epidemic. Indeed the Mondair outbreak and the Darrang epidemic have one feature in common which

throws light on the equally virulent nature of the two epidemics, viz., the 'mortality by periods'.

In the Darrang epidemic, which lasted for approximately seven weeks, there was no general diminution in mortality rates on successive periods. It was 87 per cent in the beginning, and 75 per cent towards the end of the epidemic. In the Mondair outbreak which lasted for three weeks, the case mortality was equally high at all periods of the epidemic, but the epidemic was of shorter duration and was localized.

In the Sibsagar epidemic, however, a gradual diminution in case mortality occurred among the untreated group varying from 60 per cent to 20 per cent during successive stages, whereas in the bacteriophage treated group it was the same—20 per cent throughout the epidemic. If the bacteriophage treatment then tends to make the mortality curve flat, to that extent it appears that bacteriophage may have acted in Mondair, as it did in Sibsagar and Darrang epidemics, but no comparisons can be made with an untreated group in Mondair because of the paucity of cases in this classification. It was unfortunate that no bacteriological investigations could be made during the epidemic, as the report of this epidemic was received late, but strains of vibrios collected from convalescent cases have proved atypical non-agglutinating strains.

From the 65 reported cases of cholera analysed in the body of the paper it might be judged, in spite of the question of the virulence of the infection which has been discussed, that bacteriophage treatment has given no apparent striking results. However, as has been stated previously, there had occurred during the period of this epidemic, a large number of cases of diarrhoea which had received bacteriophage. These were not reported as cholera. To ascertain whether these were actually cases of cholera, specimens of blood of six such diarrhoea cases were obtained for agglutination tests. Of these six diarrhoea cases, five gave a positive agglutination against a known strain of cholera vibrio (653) in some cases up to the titre of 1 to 100. As a control, sera of two known cholera cases were examined and proved positive in 1 to 100 in one case, and 1 to 300 in another. Cholera vaccine has not been used in this locality since 1932. It must be concluded, therefore, that these diarrhoea cases were really cases of cholera.

We realize, however, that such unreported mild cases must be present in all epidemics. The significance of this factor in evaluation of the bacteriophage treatment is discussed in another paper (Pandit *et al.*, 1936).

¹ In view of these findings it would be premature to say, in the present state of our knowledge, that bacteriophage had absolutely no effect in altering the character of the epidemic in some ways.

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IMMUNIZATION AGAINST PLAGUE WITH LIVE VACCINE.

BY

L. OTTEN,

Director, Pasteur Institute, Bandoeng, Netherlands East Indies.

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A FORMER paper dealt with the experimental results obtained with various dead vaccines in guinea-pigs, wild house rats (*R. r. diardi*), white laboratory rats, mice and monkeys (*Macac. cynomolgus*). It appeared that the nature of the experimental animal was by far more essential to the results than the nature of the vaccine used. No dead vaccine whatever conferred satisfactory protection to guinea-pigs and wild rats—the test animals most susceptible to plague infection.

I, therefore, turned to a live vaccine, just as also did Strong (1906, 1907) who, after equally disappointing results with dead vaccine, more successfully carried on his investigation with a live strain. While Strong (*loc. cit.*) used the artificially attenuated strain 'Maassen', already experimented with by Kolle and Otto (1903 *a* and *b*, 1904) some years previously, I selected a strain cultivated from a plague rat (found dead at Tjiwidej, chief town of a district near Bandoeng) which after rat-passage and storage in deep serum-agar stab-culture 4 months later casually was found to have entirely lost its virulence: subcutaneous injection of half an agar-slope culture into rats and guinea-pigs provoked no symptoms whatever. At first I intended to use another available strain, cultivated in 1920 from the bubo of a man suffering from plague in Mid-Java, and which had also been stored in deep serum-agar stab-culture since 1924. In January 1930, 10 years later, this strain 'Java' showed markedly reduced virulence; the wild rat withstood half an agar-culture, a dose, however, still lethal for guinea-pigs. One year later an entirely avirulent variant could be sub-cultured. Since, however, in some preliminary experiments its immunizing potency seemed below that of the above-mentioned strain 'Tjiwidej' I decided for this so quickly, spontaneously attenuated strain.

All experiments were carried out in exactly the same way as already described for those with dead vaccine: one subcutaneous injection (or three injections at a week's interval) of a dose expressed in fractions of an agar-slope culture after 48 hours' growth at 30°C., and subsequent subcutaneous infection 3 weeks after the (last) immunizing dose with a body strain as applied by Stevenson and Kapadia (1911), viz., 1 c.c. of a 1/100,000th dilution of 250 mg. spleen of a freshly dead

plague rat, with the difference that the dilution administered was usually 4 times higher and suspended in 0.1 c.c. instead of in 1 c.c. of the vehicle. As a rule the highly virulent strain 'Preanger' was used which is constantly stored as mentioned above. For each experiment after guinea-pig sub-passage some rats were infected and, as determined by microscopic examination, the most heavily infected spleen was selected. The number of viable and highly virulent plague bacilli of the test dose for rats averaged 50,000 and for the guinea-pigs a ten-fold dose was applied; the observation period allowed was 30 days*.

Though the first experiment (partly with 3 injections—1/50th, 1/10th and 1/5th agar-culture—and partly with one injection of 1/5th culture) gave rather satisfactory results in rats (15 : 14/1) as well as in guinea-pigs (15 : 15/0), in further experiments only 57.7 per cent of the rats (45 : 26/19) and 25 per cent of the guinea-pigs (20 : 5/15) survived, irrespective of the dosage administered and the number of injections given. As the strain had been continuously sub-cultured on agar since the first experiment, the possibility of bacterial dissociation as a cause of this lack of success was suggested: such appeared indeed to be the case. In agar-plate cultures two differently shaped colonies could be easily distinguished. According to the usual terminology these variants were designated as 'smooth' and 'rough', a distinction to be discussed later in connection with the dissociation phenomenon in *Bacillus pestis*. With these two variants which, stored in stab-culture, maintained their characteristics, extensive series of tests were carried out in rats and guinea-pigs with gradually higher dilutions of one dose only (cf. Table I).

The results obtained with these variants were rather divergent: while with the 'Tjiwidej' smooth variant in a dose of 1/5th to 1/1,000th agar-culture 75 to 100 (average 85) per cent of the rats survived, a survival rate of only 24 to 50 (average 32.5) per cent was attained with the 'Tjiwidej' rough variant. When using higher dilutions for immunization, the effect was less favourable but an equally striking difference was noted, viz., 70.8 per cent survival against 22 per cent with a vaccinating dose of 1/5,000th to 1/100,000th culture. Even with an immunizing dose of 1/1,000 millionth culture of 'Tjiwidej' smooth (containing about 5 viable bacilli) 2 out of 10 rats survived†.

In guinea-pigs the difference of antigenic potency was even more striking, though in this test animal a higher vaccinating dose was required to give optimum differential results: 80 to 100 (average 94) per cent survival with 1/5th to 1/1,000th culture of 'Tjiwidej' smooth against 15 to 30 (average 23) per cent in the corresponding rough series.

* Since sufficiently good results had already been produced in white laboratory rats and monkeys with a dead vaccine, even better results could be expected with a live vaccine in these animals. As such was indeed the case (white rats 15 : 15/0, monkeys 35 : 33/2 with a ten- and hundred-fold infecting dose respectively) I confined myself in further experiments to guinea-pigs and wild rats.

† This is the more striking since these results are superior to those with the highly virulent strain 'Preanger': of some 42 rats, which had survived infection with decreasing doses of this strain in experiments described in my former paper, after about 2 months only 2 resisted re-infection with the usual test dose of the same strain. These two rats had survived a first infection with the same test dose containing round about 30,000 viable bacilli; the remaining 40 rats all died acutely, though the first (vaccinating) infection for most of them had been more than 5 viable organisms; 10 had been inoculated with 5, 7 with 20, 6 with 30, 5 with 50, 7 with 200, 3 with 500 and 2 with 2,000 viable bacilli. This experience endorses the contention often expressed, but lacking definite evidence, that the immunizing potency of a strain does not in the first place depend on its virulence.

TABLE I.

Vaccination dose (agar-culture).	HOUSE RAT.				GUINEA-PIG.			
	Results Tjiw. smooth.	Percentage survival.	Results Tjiw. rough.	Percentage survival.	Results Tjiw. smooth.	Percentage survival.	Results Tjiw. rough.	Percentage survival.
1/5	(20) 20: 15/5	75	(20) 20: 7/13	35	(21) 21: 20/1	95	(20) 20: 3/17	15
1/25	(10) 10: 10/0	100	(10) 10: 5/5	50	(9) 9: 9/0	100	(11) 11: 3/8	27
1/50	(20) 20: 19/1	95	(20) 20: 8/12	40	(20) 20: 20/0	100	(20) 20: 5/15	25
1/100	(25) 25: 20/5	80	(25) 25: 6/19	24	(35) 35: 35/0	100	(35) 35: 11/24	31
1/500	(10) 10: 10/0	100	(10) 10: 4/6	40	(25) 24: 20/4	83	(25) 24: 4/20	16.5
1/1,000	(50) 50: 41/9	82	(50) 50: 14/36	28	(10) 10: 8/2	80	(10) 10: 2/8	20
1/5,000	(10) 10: 7/3	70	(10) 10: 1/9	10	..	..	..	..
1/10,000	(20) 19: 15/4	79	(20) 20: 5/15	25	(20) 19: 10/9	52.5	(20) 20: 0/20	0
1/100,000	(20) 19: 12/7	63	(20) 20: 5/15	25	(10) 10: 2/8	20	(10) 10: 0/10	0
1/1 ion	(20) 19: 13/6	68	(20) 20: 1/19	5	..	..	..	..
1/10 ion	(10) 10: 5/5	50	(10) 10: 0/10	0	..	..	..	..
1/100 ion	(10) 9: 1/8	11	(10) 10: 0/10	0	..	..	..	..
1/1,000 ion	(10) 10: 2/8	20	(10) 10: 0/10	0	..	..	..	..
1/5-1/1,000	(135) 135: 115/20	85.2	(135) 135: 44/91	32.5	(120) 119: 112/7	94.0	(121) 120: 28/92	23
1/5,000-1/100,000	(50) 48: 34/14	70.8	(50) 50: 11/39	22.0	(30) 29: 12/17	41.4	(30) 30: 0/30	0
1/1 ion-1/1,000 ion	(50) 48: 21/27	43.7	(50) 50: 1/49	2.0	..	..	..	..
Controls	..	90: 0/90 = 0 per cent survival.	..	80: 0/80 = 0 per cent survival.	..	..	..	..

Note.—(20) 19: 15/4 = 20 vaccinated, 19 infected (one dead before infection), of which 15 survived and 4 died.

The superiority of the smooth antigen was shown not only by the lower mortality rate but also by the longer period between infection and death. This averaged 8.5 days in the 45 rats dead in the smooth series (immunized with 1/5th to 1/10 millionth culture) against 6 days and 4.1 days in the 159 and 90 rats dead in the rough series and among the controls respectively. This difference is also present in guinea-pigs though less pronounced, as the period of infection in controls, averaging 6 days, exceeds that in rats; for the rough series it averaged 8, for the smooth series 9.4 days. In the following table the results of all the experiments that were carried out with 'Tjiwidej' smooth in doses down to 1/1,000th cultures during the years 1930-1935 are collected. Guinea-pigs inoculated with a full-agar culture served as a control of the innocuousness of the vaccine prepared with this strain for inoculation in man. Some 76 guinea-pigs injected for the same purpose with a full broth culture (5 c.c. corresponding to round about 1/5th agar-culture) cultivated from the original stab-culture are not included; of these animals 96 per cent survived (76 : 73/3).

TABLE II.

Vaccination dose.	HOUSE RAT.		GUINEA-FIG.	
	Results.	Percentage survival.	Results.	Percentage survival.
2	(10) 10 : 9/1	90		..
1	(10) 10 : 8/2	80	(97) 97 : 93/4	95.8
1/5	(20) 20 : 15/5	75	(21) 21 : 20/1	95
1/25	(10) 10 : 10/0	100	(9) 9 : 9/0	100
1/50	(60) 60 : 52/8	86.6	(30) 30 : 29/1	96.6
1/100	(340) 340 : 272/68	80	(265) 264 : 234/30	88.6
1/500	(10) 10 : 10/0	100	(25) 24 : 20/4	83.3
1/1,000	(50) 50 : 41/9	82	(10) 10 : 8/2	80
TOTALS ..	(510) 510 : 417/93	81.7	(457) 455 : 413/42	90.7
Controls ..	200 : 2/198	1.0	170 : 0/170	0.0

In rats a survival rate of more than 80 per cent and in guinea-pigs of more than 90 per cent was attained without any loss through inoculation; only a few animals died during the vaccination period from some intercurrent disease. Rats can resist even 1 to 2 agar-cultures without any ill effect, whereas the injection of the same dose of the same suspension killed by heat at 60°C. kills 35 per cent within 48 hours from toxæmia (20 : 13/7). This paradoxical phenomenon may be explained by the fact that a fresh suspension of live bacilli of this strain is but slightly or not at all toxic so that the organism can resist the endotoxins gradually produced by the slowly dying bacilli. When, on the contrary, a suspension is quickly killed by heating at 60°C., all endotoxins are produced simultaneously and the organism is unable to meet so highly toxic a dose. Besides, the possibility is suggested that the heating of endotoxins may give rise to products of an even more toxic nature. Death from toxæmia can also be provoked by a fresh live suspension provided it is

injected intraperitoneally, since by this mode of injection the bacilli are, at least partly, subject to quick destruction and the rat dies from toxæmia through the mass-production of endotoxins accordingly. That the strain 'Tjiwidej' is absolutely avirulent is clear, not only from the total absence of deaths in the experiments mentioned above but also from the following test made in a series of 4 rats subcutaneously inoculated with a full agar-culture, of which 2 were killed after 24 and 48 hours respectively and two served as controls. The strain, sub-cultured from the organs of the first two rats, was further sub-passaged in 4 rats with the same dose; even after the tenth sub-passage the controls survived in perfect health.

Some experiments were carried out to investigate the question as to whether seeding at 37°C. could further raise the antigenic potency of the strain. According to Schütze (1932) the *Bacillus pestis* possesses two antigens, a somatic antigen and one contained in the gelatinous envelope, the latter being of paramount importance for the efficiency of a vaccine. Its development should be highly favoured by growth at 37°C. The table below shows that seedings at 30°C. or 37°C. yield practically the same results; two different samples of the vaccine were used, the first one immediately after preparation and also 7 days afterwards, the other immediately.

TABLE III.

Vaccination dose.	HOUSE RAT.			GUINEA-PIG.		
	30°C.	37°C.	Controls.	30°C.	37°C.	Controls.
	Results.			Results.		
1/50 after preparation ..	10 : 7/3	10 : 9/1	} 10 : 0/10 {	9 : 8/1	10 : 8,2	} 10 : 0/10 {
1/50 7 days later ..	10 : 9/1	10 : 9/1		10 : 8/2	10 : 10,0	
1/100 after preparation ..	10 : 8/2	10 : 9/1	10 : 0/10	10 : 10/0	10 : 7/3	10 : 0/10
TOTALS	30 : 24/6	30 : 27/3	20 : 0/20	29 : 26/3	30 : 25/5	20 : 0/20
Percentage survival ..	80	90	0	90	83	0

With a view to the evaluation of the results obtained with the strain 'Tjiwidej' those of other investigators with live vaccine will be briefly summarized here. Already in 1897 Albrecht and Gohn (1900), members of the Austrian Plague Committee, who went to Bombay shortly after the outbreak of plague, immunized some guinea-pigs and rats (presumably *R. norvegicus*) with spontaneously attenuated strains and tested them after a vaccination period of some weeks. The results were fairly good but, since the strains used still caused deaths from plague and the number of experimental animals was small and, last but not least, the number of controls was absolutely insufficient, these tests are of slight value. This criticism holds good for those of Yersin and Carré (1900) also: the strain they used gave rather good results in rats (white rats?) but it also caused deaths from plague. This disadvantage could be met by using older cultures of 40 to 50 days' growth,

at the expense however of the results attained ; also the number of controls used in their few experiments was too small.

Of more value are the investigations of Kolle and Otto (*loc. cit.*) made in extensive series of experiments with different strains, partly spontaneously and partly artificially attenuated. The spontaneously attenuated strains, however, often caused buboes and occasionally deaths from sub-chronic plague. The artificially attenuated strains—all derived from the so-called strain 'Maassen' [after Maassen (quoted by Kolle and Otto, *loc. cit.*) who attenuated this strain in a way still unknown] also caused deaths from plague in guinea-pigs. Only the strain 'Maassen V', grown at a temperature of 40°C. to 41°C. over a long period, was absolutely avirulent for guinea-pigs even when injected subcutaneously in a dose of 1 to 2 agar-cultures, a dose able to kill them from toxæmia only when injected intraperitoneally. Whether the cause of death in rats must be accounted for by plague or by toxæmia, is not clear from the text. Results were more favourable in guinea-pigs than in rats, the survival rate for the former averaging 72, for the latter only 35 per cent.

The most accomplished research work was carried out by Strong (1906, 1907) some years later with the same strains 'Maassen alt' and 'Maassen V':. Numerous experiments were made in guinea-pigs and monkeys (*Cynomolgus philippinensis*) without any deaths from plague notwithstanding a vaccinating dose of 1 to 2 agar-cultures. This suggests the possibility that these strains underwent further spontaneous attenuation in the course of time ; the few animals lost during the vaccination period apparently died from toxæmia. The results obtained by Strong (*loc. cit.*) surpassed by far those of Kolle and Otto (*loc. cit.*). It must, however, be noted that his experiments are not exactly comparable with those of the latter investigators who usually chose a smaller vaccinating dose and allowed a longer vaccination period in many of their experiments. The fact remains that Strong, considering the marked difference between the effect with live and dead vaccine, rightly concluded that vaccination with live bacilli deserves preference. After having stated the innocuousness of the strain 'Maassen' for man by inoculation of some 200 persons with this vaccine, that even in a dose of a whole agar-culture did not give rise to any severe reactions, Strong expressed himself as a convinced supporter of vaccination with live vaccine. It was not until a quarter of a century later, however, that its application was realized.

In 1927 Pirie carried out some experiments in Gerbilli (*G. lobengulæ*) with a view to investigating whether immunization with live avirulent plague bacilli was more efficient than with a dead vaccine, which had given rather unsatisfactory results. Of the 4 strains he used one appeared to have maintained its full virulence : all test animals vaccinated with it died acutely from plague. Though they did not cause deaths from plague the 3 other strains nevertheless gave a high mortality rate during the vaccination period, presumably from toxæmia. When tested by infection after 9 days, one strain appeared an absolute failure and the other two gave survival rates of 33.3 and 50 per cent respectively, a result, though superior to that with dead vaccine, not altogether satisfactory in view of a percentage mortality from toxæmia of 20 to 25.

Finally, almost at the same time, two communications were published on this subject. one by Girard and Robic (1934) (read by Mesnil before the

TABLE IV.

Author.	Strain.	Test animal.	Dose (agar-culture).	VACCINATION.		INFECTION.		CONTROLS.	
				Results.	Percentage loss.	Results.	Percentage survival.	Results.	Percentage survival.
Kolle and Otto ¹ ..	'Maassen alt'	Guinea-pig	1/100-1/20	32: 23/9	28	22: 17/5	77.3	37: 0/37	0
	" V	"	1/10-3	26: 26/0	0	21: 14/7	66.6		
	" alt	White rat	1/100-1/10	20: 20/0	0	19: 8/11	42.1	31: 1/30	3.2
	" V	"	1/10-1/2	20: 15/5	25	15: 4/11	26.6		
Strong ..	'Maassen alt'	Guinea-pig	1	30: 28/2	6.6	28: 25/3	89.3	70: 0/70	0
	" V	"	1	51: 51/0	0	51: 35/16	68.8		
	" alt	Monkey	1/2	49: 43/6	12	43: 30/13	70	80: 7/73	8.7
	" V	"	1/2	43: 42/1	2.3	42: 21/21	50		
Otten ..	'Tjiwidej' sm.	Guinea-pig	1/1,000-1/100	300: 298/2	0.6	298: 262/36	87.9	180: 0/180	0
	"	"	1/50-1	233: 233/0	0	233: 224/9	96.1		
	"	House rat	1/1 ion-1/100	460: 457/3	0.6	457: 363/94	79.4	200: 2/198	1
	"	"	1/50-2	110: 110/0	0	110: 94/16	85.4		

'Académie de Médecine' in Paris in the session of 26th June, 1934), the other by me (Conference of the Netherlands' Association for Tropical Medicine in Amsterdam on 25th March, 1934). Girard and Robic used a strain, designated 'E. V.' and isolated in 1926 from a human case of bubonic plague in Madagascar, since grown at room temperature (18°C. to 25°C.) and sub-cultured monthly on agar. Six years later, in 1932, this strain appeared to be absolutely avirulent for guinea-pigs when injected subcutaneously in a dose of a whole agar-culture; white mice and rats, however, could resist only a relatively small dose (not exactly indicated by the authors); with increased doses they died from toxæmia just as did 20 to 30 per cent of guinea-pigs when inoculated intraperitoneally with $\frac{1}{3}$ rd agar-culture or more. However, their experience that guinea-pigs, when vaccinated with this strain, showed to be absolutely immune against cutaneous infection with virulent plague matter (spleen, lung, etc.), was of paramount importance.

To facilitate a general view on the subject the results of Kolle and Otto—as far as immunization by subcutaneous injection with a live avirulent vaccine is concerned—of Strong and mine are collected in the synoptic table above. The experiments of Pirie are only few in number, while those of Girard and Robic cannot be quoted here because of particulars not being mentioned and data about number of test animals being lacking.

The results obtained with the strain 'Tjiwidej' undeniably compare favourably with those with the strain 'Maassen', especially when the far smaller dose applied in the majority of the test animals and the fact that immunity is less easily produced in wild rats than in white rats and monkeys, are considered.

As to particulars about the immunity produced by the smooth strain 'Tjiwidej' I investigated into the period required for its development to a maximum and into the period this was maintained. In Table V the results of all experiments in rats and guinea-pigs, infected in the usual way 1 to 21 days after immunization with a dose of 1/100th agar-culture, are collected; the figures represent the total of 5 series in rats and of 3 in guinea-pigs:—

TABLE V.

Infection after number of days.	DEVELOPMENT OF IMMUNITY IN			
	HOUSE RAT.		GUINEA-FIG.	
	Results.	Percentage survival.	Results.	Percentage survival.
0	50 : 0/50	0.0	30 : 0/30	0.0
1	40 : 13/27	32.5	30 : 1/29	3.3
2	40 : 9/31	22.5	29 : 4/25	13.7
3	45 : 16/29	35.5	30 : 3/27	10.0
5	45 : 25/20	55.5	29 : 13/16	44.8
7	45 : 23/22	51.5	30 : 14/16	46.6
10	45 : 30/15	66.6	29 : 24/5	82.7
14	50 : 42/8	84.7	26 : 23/3	88.4
21	49 : 43/6	87.7	30 : 27/3	90.0

It appears that an appreciable degree of immunity has already developed within a few days, especially in rats, which, however, becomes significant only after some 5 to 7 days and gradually increasing does not reach its maximum until 2 to 3 weeks. This does not tally with the experience of Stevenson and Kapadia (1911), who noted that immunity developed quickly in rats after injection of $\frac{1}{4}$ c.c. of the Haffkine vaccine, reached its maximum after 2 to 3 days to drop, though slowly, after that period. It must be borne in mind, however, that the Haffkine vaccine confers on the rat a relatively low degree of immunity, widely differing in consecutive series of experiments (in their first experiment 28.2 per cent survived after 3 days, in their second 62.5 per cent) and simultaneous tests may give divergencies amounting to even 100 per cent. It is obvious, therefore, that definite conclusions as to the development of immunity can hardly be drawn from their results. In my opinion, a gradual increase of immunity as shown in my experiments with the strain 'Tjiwidej', that agrees with the experience of Yersin and Carré (*loc. cit.*) just as with that of Girard and Robic (*loc. cit.*) and with the gradual appearance of anti-bodies demonstrable *in vitro*, such as agglutinins, is more in harmony with the facts.

In this connection it is a matter of doubt as to whether the immunity noted in the first days after vaccination be of an exclusively specific nature; especially the fact established by Stevenson and Kapadia that infection some few hours after vaccination gave a higher survival rate against that of controls, suggests that non-specific factors play a part, viz., an aspecific increase of resistance as presumed by Pfeiffer and Issaëf (1894). Anyhow it is clear from the experiments of Stevenson and Kapadia and from mine that a so-called 'negative phase' is not shown in accordance with the experience gained in man. From the data collected by Bannerman (1901-1902) it appears that mortality in those inoculated with the Haffkine vaccine is always lower than in those not inoculated, even in the last days of the incubation period and even when the first symptoms of the disease appear on the day of vaccination or are already manifest at that moment. In vaccination of man with live vaccine a negative phase could not be observed either, as the data concerning this subject will show.

As to the duration of immunity, this has been ascertained in rats and guinea-pigs. The wild rat, however, proved an unsuitable test animal in this respect, since, when imprisoned in small cages of 25 cm. by 12 cm. by 12 cm., it begins to languish after some months so that an increasingly higher loss of rats occurs after some 4 to 5 months. The experiments with rats, therefore, were discontinued after 6 months; though guinea-pigs were more suitable, the test with them could not be extended over more than 9 months. In Table VI the results are collected: the vaccinating dose was 1/50th agar-culture, partly inoculated by one and partly in 3 injections at a week's interval; 3 weeks after the last injection the first series was infected with the usual test dose (ten-fold dose for guinea-pigs), 3 months later the second series and so on.

After 6 months the immunity of rats had decreased considerably; their bad health, however, must be taken into consideration. In guinea-pigs, too, immunity had appreciably dropped: after 6 months it was still fairly satisfactory, after 9 months, however, it had markedly decreased. That it has not yet entirely faded away appears from the lower mortality rate as compared with controls (75 per cent against 100 per cent) and from the longer period between infection and death (8.2

against 6.1 days). As a matter of course these experiments have little or no practical significance for the solution of the question of how long immunity may persist in man; this can be solved only by the course of an epidemic after vaccination.

TABLE VI.

Infection after	DURATION OF IMMUNITY IN					
	HOUSE RAT.			GUINEA-PIG.		
	VACCINATED.		Controls.	VACCINATED.		Controls.
	1 injection.	3 injections.		1 injection.	3 injections.	
	Results.			Results.		
3 weeks ..	10 : 8/2 ₁₃	10 : 10/0		10 : 2/8 _{3.6}	10 : 9/1 ₈	
3 months ..	10 : 7/3 _{5.7}	10 : 6/4 ₈	10 : 0/10 _{3.8}	10 : 8/2 _{17.5}	10 : 10/0	10 : 2/8 ₁₀
6 " ..	10 : 2/8 _{3.6}	10 : 3/7 _{3.6}	10 : 1/9 ₃	10 : 6/4 _{9.5}	10 : 8/2 _{11.5}	10 : 0/10 _{6.3}
9 " ..	..	..	..	10 : 2/8 _{8.3}	10 : 3/7 _{8.1}	10 : 0/10 _{6.1}

The subscript figures indicate the average number of days of survival after infection.

VACCINATION IN MAN.

Before this vaccination could be started, it obviously was indispensable to prove the suitability of the strain 'Tjiwidej', not so much as to the possibility that it occasionally causes death in man—such being rather improbable considering the fact that guinea-pigs and rats resist inoculation of a whole agar-culture without any untoward effect—as to ascertain the nature of the reaction in response to the injection. Animal test had shown that as a rule in guinea-pigs, but rarely in rats, a well-marked infiltration develops at the site of injection. Generally this infiltrate is resorbed in a few weeks, sometimes, however, it gives rise to a sterile abscess that finally breaks without any disadvantage to the guinea-pig. If such infiltrates, or abscesses should regularly develop in man too, this would mean a conclusive objection to the application of this strain for the vaccination of the population. Fortunately the vaccine appeared quite harmless to man, as the reaction was slight beyond expectation: after inoculation of 1/50th culture (about 100 millions viable and 200 millions dead organisms) the reaction was confined to a small infiltrate with slight, if any, rise of temperature, even when the dose was increased to 1/25th culture. After some more volunteers had submitted to vaccination and had not

shown any serious local or general symptoms even with an increased dose up to $\frac{1}{4}$ th culture, the start of a preliminary immunization of the population according to the alternating system was indicated in order to decide whether this vaccine was able to protect man against plague infection.

During the epidemic in Mid-Java, in 1921-1922, with the Haffkine vaccine prepared partly in the Haffkine Laboratory at Bombay and partly in the Pasteur Institute at Batavia, according to this system, an extensive experiment was made. More than 35,000 persons were vaccinated during those years: in each household half of the inmates were selected as far as possible according to age and sex in order to obtain an exactly comparable control group. This procedure resulted in a reduction of plague mortality to 50 per cent, undoubtedly an appreciable reduction but not sufficient to make an impress on the minds of the native population, the less so since in 1922—the year of the greatest number of vaccinations (80 per cent)—in some sub-districts, where the epidemic showed an acute rise, mortality was reduced by only $\frac{1}{3}$ rd. These results obviously compare unfavourably with those obtained in British India, where according to many communications on the activity of the Haffkine vaccine a six- to ten-fold reduction of the mortality rate and even more is recorded. Most of these reports, however, are of little or no statistical value whatever, as was already emphasized in a former paper.

The usual mistake met with is that vaccination is confined to those willing to submit to it and that the incidence of plague in this group is compared with that amongst those opposed to immunization. As a matter of course the last group consists mostly of people of little or no education at all or contains adherents of some special creed, living accordingly under particular conditions. In British India especially with its strict caste system, the standard of living and the housing conditions will widely differ. From this it will be clear that, as a rule, the two groups are far from comparable. A further statistical error arises from the circumstance that vaccination may proceed slowly and is not completed until the end of the epidemic. During its increase—in the period of highest mortality—the victims mainly prevail among unvaccinated; consequently results will be the more flattered the later vaccination is started and the more slowly it proceeds. Even when vaccination is started opportunely, statistics may be flattered if plague does not spread diffusely but is more or less localized and those vaccinated may, for the greater part, dwell in the slightly or not infected quarters. In short, in almost none of the available plague statistics has the erratic spread of plague been taken into consideration and, as a rule, wholly heterogeneous groups have been compared. To ensure reliable statistics an exactly comparable control group is required and this is ensured only by the alternating system of vaccination in order to prevent volunteers from being opposed to those who refuse, as well as a small group of vaccinated from being compared with a large one of unvaccinated available from different phases of the epidemic and living in different quarters. This is feasible only by an equal partition of the population according to sex and age up to its smallest social unit, the household. Only in this way are two similar groups ensured, living under the same conditions and exposed to the same risk of infection during the same period. Though the preliminary measures as well as the execution of such an alternating vaccination scheme require a great deal of time and effort, it must be emphasized that only this method guarantees two exactly comparable groups in order that a marked difference of the mortality rates in favour of those

vaccinated can be explained only by their artificially increased resistance owing to vaccination.

The preliminary vaccination mentioned above was carried out in two sub-districts of the Regency of Bandoeng, viz., Bandjaran and Batoedjadar. After registration of the houses and their inmates of all 25 villages (desas), 37,000 persons were alternately vaccinated in the course of one month (from 3rd November to 6th December, 1934). This vaccination in each village and its accessory hamlets, forming a unit, was accomplished in one day unless too scattered. After it was finished full attention was paid to all cases of death since, apart from the diagnostic difficulties, it is impracticable to trace all living cases of plague. In all deaths recorded spleen and lung puncture was performed and smears were made of both, which in addition to spleen matter were sent to the Pasteur Institute, where the diagnosis was ascertained by microscopic examination and, in doubtful cases, by animal test. Over a period of 5 months (21 weeks) more than 1,200 cases of death were checked in this way and 356 cases of plague detected; the partition of these cases among vaccinated and unvaccinated is shown in Table VII:—

TABLE VII.

Sub-district.	BUBONIC PLAGUE.					
	VACCINATED.			UNVACCINATED.		
	Number.	Deaths.	Mortality per mille.	Number.	Deaths.	Mortality per mille.
Bandjaran ..	18,479	28	1.5	20,669	103	4.9
				*2,755	10	3.4
				17,914	93	5.1
Batoedjadar ..	18,956	10	0.5	24,088	110	4.6
				*2,519	3	1.2
				21,569	107	5
Totals ..	37,435	38	1.01	44,757	213	4.75
				*5,274	13	2.46
				39,483	200	5.05

*Absentees from causes other than disease, childbirth or old age.

The cases occurring in the first week after vaccination (21, of which 4 were among the vaccinated) are eliminated since, on the one hand, these cases concern vaccinated people in whom immunity had not yet fully developed, so that their death from plague cannot be considered as any reflection on vaccination; when adding them to the number of failures the efficiency of the vaccine would be unjustly minimized. On the other hand, deaths from plague among those not vaccinated is partly due to persons falling ill just before vaccination; if these were added to the statistics, the effect of vaccination would be flattered. Cases of primary pneumonic plague are omitted also (84 cases, of which 36 were among the vaccinated) on the presumption, based upon data available from literature and personal research, that this vaccination could not be expected to guarantee any protection against ærogenic infection, at least when subcutaneously administered at the relatively small dose adhered to till now*.

The total number of deaths from bubonic plague ascertained in the 21 weeks period mentioned above amounted to 251, of which 38 occurred in vaccinated and 213 in the control group, giving a mortality rate of 1.01 against 4.75 per mille or a reduction of mortality in vaccinated to almost 20 per cent. On closer examination of the particulars of both the sub-districts, however, it appears that they are widely divergent. In Bandjaran mortality is reduced to only 30 per cent (1.5 : 4.9) against almost 10 per cent in Batoedjadjar (0.5 : 4.9). This striking contrast must be explained by unequal prevalence of pneumonic plague in these two sub-districts. In Batoedjadjar pneumonic plague was confined to some local foci, the number of cases amounting to only 16; in Bandjaran, however, shortly after vaccination was finished, a violent outbreak was recorded in one village giving rise to dissemination in the neighbourhood that could be checked only after some months. The total number of cases amounted to 68, i.e., more than 4 times that of Batoedjadjar. These figures relate exclusively to those cases which could be clinically or epidemiologically ascertained; as a matter of course, in addition to these, many cases will have occurred that could not be differentiated. As a rule the informants are not able to discriminate incidental cases of primary pneumonic plague from bubonic plague. Microscopic examination is not conclusive either, since smears typical of primary pneumonic plague in the absence of plague bacilli in the spleen are as rare as is the reverse in bubonic plague: between these two typical findings numerous transitions exist, differences being merely quantitative and so their interpretation must necessarily be arbitrary. These sporadic and undetected cases of primary pneumonic plague will range among those of bubonic plague and, since pneumonic plague will prevail equally in both groups, it is obvious that even a small number of these cases is apt unfavourably to affect statistics of bubonic plague, i.e., of a disease the incidence of which markedly decreases among successfully vaccinated as compared with non-vaccinated people.

* After the violent epidemic of primary pneumonic plague in Manchuria (1911-1912) Strong endeavoured to immunize a great number of monkeys against ærogenic infection with the same avirulent strain as used in his former plague experiments: over 90 per cent of them died from pneumonic plague. Guinea-pigs inoculated with the strain 'Tjiwidej', when dying after a prolonged period of illness, as a rule show symptoms of secondary pneumonic plague, the spleen on the whole having a normal aspect and repeatedly yielding a negative result on microscopic examination and also occasionally even when sub-cultured. In immunized rats that die ten days or more after infection this syndrome is usually found too, thus supporting the fact that even in immune test animals the lungs remain a *locus minoris resistentiæ*.

Apart from the unfavourable influence of overlooked cases of pneumonic plague in the sub-district Bandjaran, the effect of this trial is minimized by two further factors: in the first place by the relatively high number of absentees from causes other than disease, childbirth and old age. This group mainly concerns contract-coolies of both sexes, adults and children, who are periodically employed away at the neighbouring estates. Since, however, these estates, as a rule, are not infected, this part of the population runs no risk of infection during its absence from home, so that a lower mortality from plague may be expected among them. This indeed appears to be the case, as plague mortality amongst them was half of that among those not vaccinated, who stayed at home uninterruptedly; this means a dilution of the statistical material in favour of the unvaccinated. When these absentees to a number of more than 5,000 persons are eliminated, mortality in the control group increases to 5.05 per mille, i.e., amounts to five-fold that in vaccinated; for Batoedjadar only mortality increases to 5 per mille, exactly 10 times that of those vaccinated.

Another factor claiming attention is the different age distribution of children in both groups and its reflection on the mortality rate*. It appeared that the average age of the vaccinated children was much higher than that of those serving as controls. In the former group the percentage of children in the age-group of 1 to 5 years amounted to only 23 against 59 in the latter. Though equal distribution according to age was devised, the greater portion of this age-group obviously escaped vaccination, probably on account of the natural inclination of the vaccinator to evade trouble and screaming. Sticker (1910) has already drawn attention to the fact that according to the data available in Bombay babies are but exceptionally infected with plague and that infection in infants under 6 years is scarcer than in older children. These data, however, are but little convincing since they concern hospital cases and it was not ascertained that the age distribution of those attended to in hospital agreed with that of those suffering from plague outside the hospital. As regards the low plague mortality of babies, this was already confirmed in the first year of the outbreak of plague in Java in 1911. Among round about 3,500 babies of both the sub-districts now concerned not a single case was noted during the period of observation. As babies were excluded from vaccination, they are eliminated from those not vaccinated.

From the statistics of these two sub-districts it further appears that mortality among unvaccinated children of 1 to 5 years was actually lower than among those 6 to 15 years old, viz., $34/9,693 = 3.5$ per mille, against $39/6,735 = 5.9$ per mille (in vaccinated children the mortality rates were $1/2,916 = 0.34$ per mille and $9/9,762 = 0.92$ per mille respectively). The risk of infection, therefore, is markedly higher in children of 6 to 15 years. Since almost 60 per cent of the children of this age-group were vaccinated, the mortality rate of vaccinated must obviously be higher and that of unvaccinated lower than would be expected with an equal age distribution. The results of vaccination, therefore, are also unfavourably influenced by this incongruity.

* As to the age distribution it was noted during former epidemics in East- and Mid-Java, that mortality in both sexes was equal, at least in the country. In West-Java too this distribution appeared to be the same: a mortality of 4.81 per mille in males and of 4.71 per mille in females.

Anyhow, in my opinion, the results obtained in Batoedjadjar, where conditions were not distorted by the incidence of pneumonic plague, may be considered as normal and a reduction of mortality to 10 per cent as attainable, except for the interference of pneumonic plague. It is obvious, however, that such a reduction never will be realized, since it implies complete vaccination of the whole population which must be considered impracticable. When only 10 per cent fails, it already means a doubling of mortality or a reduction to only 20 per cent. Besides a single injection of a relatively small dose ($\frac{1}{5}$ th agar-culture = 3 milliards plague bacilli, of which $\frac{1}{3}$ rd is viable, for adults and half of this dose for children) guarantees protection over only a limited period roughly estimated at about 6 to 8 months. Mortality in a vaccinated area may, therefore, be expected to increase gradually till immunity be restored to its original or to an even higher level by re-vaccination. Taking into consideration all the conditions enumerated here a reduction of the mortality rate to $\frac{1}{4}$ th may be regarded as the highest actually attainable.

In the beginning of 1933 Girard and Robic carried out a preliminary test vaccination in Madagascar with the avirulent plague strain 'E. V.' mentioned before, after having checked its innocuousness for man by subcutaneous inoculation of a number of lepers. In the first 3 months 1,600 volunteers were vaccinated and 13,000 from September to January 1934—the period of seasonal prevalence—without any untoward effect being noted. From October to December 1934, about 47,000 persons were immunized against about 60,000 serving as controls. In the course of the following 5 months 22 cases of plague were recorded in those vaccinated against 100 in the control group, viz., a mortality rate of 0.47 against 1.66 per mille, i.e., a proportion of round about 1 : 3 when eliminating cases of primary pneumonic plague, which were confined to those not vaccinated. Since this vaccination was not carried out according to the alternating method, Girard and Robic are well aware of the fact that the two groups are not exactly comparable. They presume, however, that the greater part of those vaccinated dwell in the more heavily infected areas in the country and of those of the control group, on the contrary, in the far less infected centres. The reduction of mortality would, therefore, in their opinion, be far from flattered and would actually be higher than $\frac{2}{3}$ rds. This supposition may be right but it is highly possible that, in the heavily infected villages, many people abstained from vaccination, the more so since at times vaccination was available only at great distances from their dwellings. Considering that the 122 plague cases mentioned above concern only 85 of the 1,555 scattered villages, it would have been preferable if the comparative calculation of these cases had been confined to the population of the infected 85 villages only. Even then, however, exactly comparable mortality rates would not have been guaranteed, in case many families or even entire villages might have refrained from vaccination and in case the epidemic might not spread diffusely but was rather localized. Anyhow Girard and Robic are convinced that they achieved far better results with this live vaccine than with several dead vaccines in former years, which always yielded disappointing effects in test animals as well as in man.

The general vaccination of the population was here started in the last week of January 1935 in four centres of the Preanger with four squads at a time,

consisting of a native physician and four qualified nurses (mantris). At each centre the number of people daily vaccinated averaged 2,500 and if feasible, in each village, vaccination was completed in one day. Beginning in those sub-districts where plague had claimed the greatest number of victims, almost the whole area of the Preanger in which plague prevailed, comprising about 2 million inhabitants, was vaccinated in the course of the year 1935. At the end of November, when vaccination had to be discontinued on account of the Mohammedan fasting month (Ramzan), the number vaccinated amounted to round about 1,875,000, comprising more than 94 per cent of the population. Besides re-vaccination was started in some villages in which plague showed an increase after a lapse of time; to this re-vaccination also more than 90 per cent of the population submitted. Moreover, in October vaccination was carried out in some few foci of plague left in Mid-Java. The total number of vaccinations in 1935 amounted to 2,082,281, that of re-vaccinations to 236,056.

As for the results of this general vaccination it is impossible to estimate the reduction of mortality attained, due to the lack of exactly comparable controls; this can, however, be approximated by comparing the mortality rate of those vaccinated with that of those not vaccinated. The latter, comprising round about 10 per cent of the population, as a rule were absent not because of their being unwilling to submit to vaccination but because they were elsewhere engaged (market, planting, funeral, etc.); the non-appearance of an entire family occurred only exceptionally. The mode of living of those vaccinated and unvaccinated was in the main exactly the same and an equal risk of infection may, therefore, be presupposed for both groups that differ only in numerical strength.

Supposing that those vaccinated comprise 9/10ths of the whole population, it is clear that a reduction of the mortality rate to 10 per cent means a proportion between the number of cases of bubonic plague among those not vaccinated to that among vaccinated of 1: 0.9, a reduction to 15 per cent a proportion of 1: 1.35, to 20 per cent of 1: 1.8, to 25 per cent of 1: 2.25 and so on. A proportion of the number of deaths of 1: 9 would mean an equal mortality in the two groups, due to inefficacy of the vaccine or to complete loss of immunity. In the 24 sub-districts of the Regency of Bandoeng which had been successively vaccinated, 3,990 cases of plague were recorded in 1935: 2,680 before or during vaccination, 96 in the first week after vaccination (of which 51 had been vaccinated) and 1,214 more than 7 days after vaccination, viz., 475 among unvaccinated* and 739 among vaccinated. The proportion of the number of deaths in the two groups was 1: 1.55, which means a reduction of the mortality in vaccinated to 17 per cent.

This reduction fairly approaches that attained in the preliminary alternating test vaccination in the sub-district Batoedjadar (10 per cent), considering that primary pneumonic plague is included. The difference can be further explained by the rather rough manner in which this percentage is calculated, seeing that the percentage of vaccinated, averaging 90, ranged between more than 95 and hardly 80 in different villages. In a village with a percentage vaccination of 95 and

* Those not re-vaccinated have been ranged among the unvaccinated, when re-vaccination has taken place in their village.

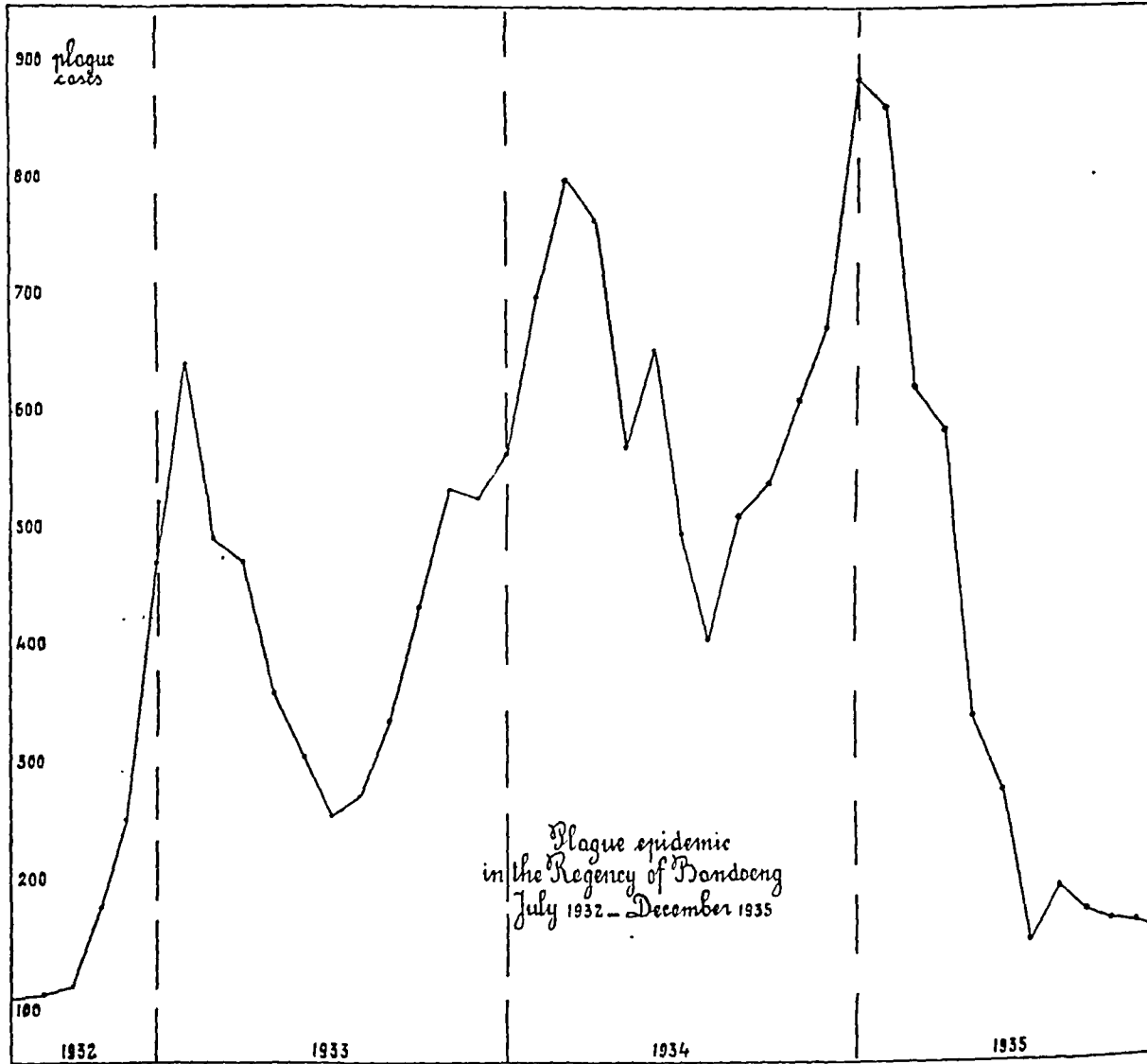
a reduction of mortality to 10 per cent the proportion between the number of deaths in vaccinated and unvaccinated is not 1 : 0·9 but 1 : 1·9, in a village with a percentage vaccination of 80 it is 1 : 0·4. When, however, calculated on the base of a 90 percentage vaccination mortality would be in the first case reduced to only 21 per cent, thus amounting to twice the actual mortality, in the latter case to 5·5 per cent, i.e., to half of the actual mortality. So it is clear that with a calculation of the reduction on the base of an average vaccination percentage of 90, results will be flattered in those villages where this percentage is lower and minimized in those where it is higher. This drawback might be partly obviated by the determination of the number of deaths in each village separately on the base of the actual percentage of vaccinated but, apart from the figures growing too small, even then exactly reliable results would not be guaranteed, since the percentage vaccination of the accessory hamlets of one and the same village widely differs so that through the localized character of plague this average percentage cannot give a satisfactory correction neither. By the above calculation I, therefore, merely endeavoured to make it plausible that the favourable results of the preliminary test vaccination (the statistical value of which is beyond doubt) are also attainable in mass-vaccination.

This conclusion is further substantiated by the suggestive course of the plague epidemic in the Regency of Bandoeng as compared with that of the last years. The incidence of plague in this area shows a typical, remittent character exactly corresponding to that of all Java as already described in many other papers: an increase at the beginning of the third quarter, the driest season, with its acme in the months round about the end of the year and a decrease half-way through the wet monsoon with the minimum in the middle of the year. While in the years 1932-1933, 1933-1934 and 1934-1935 this rise was regularly noted with an increasingly higher peak and smaller decline, in 1935 not only a quicker and steeper decline occurred but the curve was maintained on this low level for months and not until December was a slight rise noted (*vide* Graph). In connection with this rise stress must be laid upon the fact that during this month, in which the Mohammedan Ramzan occurred, re-vaccination was almost stopped; besides there prevailed many small outbreaks of primary pneumonic plague, against which vaccination is powerless.

Anyhow, the results obtained may be considered as rather satisfactory, since the vaccination as a plague repressive measure answers the aim in view, viz., the saving of human lives. Emphasis, however, must be laid on the difference between bubonic plague and other infectious diseases. In the latter, spread through contact from person to person, immunization of man may so deprive the infecting agents of their suitable medium as to cause these diseases to decrease even to vanishing point. In plague, however, not man but the rat is the originating source, human plague is only secondary to rat plague, which spreads undisturbedly despite immunization of man and is liable to prey on an increasingly greater number of victims as soon as immunity is on the wane. Though immunity may be restored by re-vaccination, this measure will, in the course of time, fail to guarantee success because of people being fain to abstain in increasingly greater numbers from periodical re-vaccination. Great advantage as the vaccination with a live vaccine in the repression of plague in man may be, the improvement of housing conditions remains indispensable and must be maintained as the only rational preventive

method capable of affecting the living conditions of the rat and of eradicating rat plague, the root of this evil.

GRAPH.



MICROBIC DISSOCIATION AND LOSS OF VIRULENCE.

The behaviour of the strain 'Tjiwidej' draws attention to loss of virulence under cultural conditions, a phenomenon characteristic of all pathogenic bacteria and already repeatedly described in connection with *Bacillus pestis*. During the first epidemic in Hongkong Yersin (1894) already established the fact that the

plague bacillus may easily lose its virulence: observing its growth on peptone-agar he noted that, after a few days, some colonies characterized by their size and higher rate of growth, when sub-cultured, appeared to have but slight if any virulence left for guinea-pigs. In connection with these findings Yersin suggested the possibility of immunization with such strains.

This experience of Yersin closely touches the problem of microbic dissociation. Until recently, however, according to general opinion, a culture was considered to consist of organisms all equally virulent and all of which shared equally in decrease and loss of virulence. It was not before 1927 that Hadley, the protagonist of microbic dissociation, opposed this conception in a general sense and threw full light on this problem, referring to numerous investigations: in the first place to those of de Kruif (1921, 1922) in *B. lepi-septicus*, one of the members of the *Pasteurella* group. De Kruif distinguished two types of colonies and stated that virulence was associated with the normal or 'smooth' type and not or only in a slight degree with the abnormal or 'rough' type. The scarcer the smooth type and the more preponderant the rough, the greater was the decrease of virulence, which was entirely lost as soon as the culture had become entirely of the rough type.

Pirie (1929) in an exact investigation into the dissociation phenomenon was the first to prove that these characteristics were also demonstrable in plague bacilli. In a sub-culture of a 2½ year old strain he distinguished, along with the normal type, designated as 'smooth', another type marked as 'rough'. The former appeared to be rather virulent, the latter absolutely devoid of virulence for rats and guinea-pigs. Contrary to de Kruif, who found that vaccination with the avirulent rough type of *B. lepi-septicus* was able to protect test animals against subsequent infection with a virulent strain (one experiment with two rabbits only!), Pirie's experiments with the rough type of *B. pestis* yielded a negative result, so that he was inclined to deny all immunizing value to this variant.

Bessonowa and Lenskaja (1931) found, among 150 plague strains, a few giving a diffuse turbidity when growing in broth. Seeded on agar-medium one of them showed a form of colony distinctly different from the normal type, also available on the same plate. The normal type was of the same virulence as the original strain, whereas the variant with its atypical growth appeared to be avirulent. In another strain, also giving diffuse turbidity in broth, the normal appearance of the colony was maintained and its virulence did not stand behind that of the original culture. In the opinion of Bessonowa and Lenskaja (*loc. cit.*) this atypical variant must be considered as the smooth type and the typical one—with agglutinative growth in broth—as rough; thus in plague bacilli (just as in *B. anthracis*) not the smooth but the rough type would be associated with virulence.

Though this contention agrees with the conception adhered to by Hadley (1927), I must oppose it. Arkwright (1921), who was the first to make these distinctions, based this denomination on the coli-typhoid-dysentery group in which the normal, freshly isolated virulent type is indeed characterized by growth in round, smooth and shining colonies, while the colonies of the abnormal type, which has little or no virulence, are conspicuous by their irregular form and dim and rough surface. Arkwright rightly designated the normal, original type as 'smooth'

and the abnormal variant as 'rough'. This, however, does not imply that this purely morphological distinction is applicable to all species of bacteria. On the contrary there are several bacteria giving rise to different types which cannot be distinguished on the base of this classification. To these species also belongs the *B. pestis*, whose normal growth departs from the usual one and which, contrary to most bacteria, shows no homogeneous turbidity, when grown in broth. However, it is illogical to designate the normal virulent type as a rough variant, which has nothing in common with it and, on the contrary, is characterized by the loss of many biochemical and antigenic properties.

This morphological distinction must, obviously, give rise to confusion, when generally applied. The more so since the cultural behaviour of many pathogenic bacteria—especially of the *B. pestis*—largely depends on the nature of the culture medium. Apart from the undeniable influence of different factors such as the peptone percentage, the degree of humidity, the pH of the medium, etc., many other circumstances unknown at present may also play a part. That is the weak point in the conception of the relation between bacterial cyclogeny and microbic dissociation as presumed by Hadley, based as it is almost exclusively upon cultural conditions, often so unfavourable to the organisms that many of the alterations observed must be considered as symptoms of atrophy and degeneration and must be ascribed to mutilation rather than to mutation, as is so aptly observed by van Loghem (1929, 1930).

That confusion already prevails is clear from the fact that according to Pirie (1929) the normal virulent type of the plague bacillus, characterized by colonies he called 'smooth', gives turbidity when grown in broth, whereas Bessonowa and Lenskaja (*loc. cit.*), on the contrary, consider—rightly—growth in broth without turbidity as normal but designate the colonies of this virulent type as 'rough'. The aspect of the smooth and the rough type as shown by their photographs exactly agree with the types observed by me, with the reservation that the normal type, growing in broth without causing turbidity and agreeing with their rough type, is called 'smooth' by me and conversely their 'smooth' is 'rough' with me. Burgess (1930) confines himself in the discussion of this phenomenon to a morphological description of the colonies. He distinguishes 3 types: small colonies with a fringe—the normal type—small round colonies with only a little, if any, fringe and larger colonies with irregular form. Vedder's (1932) classification is also based on such distinctions; his F, G and D types of colonies of *B. pestis* and pseudotuberculosis, however, do not exactly answer to the description of Burgess and even less to those of Bessonowa and Lenskaja.

Anyhow, the most important conclusion to be drawn from these divergencies is that the degree of virulence—whatever relation to antigenic structure may exist—cannot be accounted for by the morphology of the colony. Whereas Pirie stated a marked difference of virulence between his two types, Burgess' experience with his 3 types was not suggestive of such a differentiation and according to Bessonowa and Lenskaja atypical growth was associated with loss as well as with maintenance of virulence. By my investigations, too, no direct parallelism between morphology and virulence was established: both variants of the strain 'Tjiwidej' were absolutely avirulent notwithstanding that the type, which possessed highly antigenic potency, did not differ as to its growth in any respect from a typical virulent strain.

That the number of possible variants is not exhausted with those mentioned here, I experienced to my disadvantage. Returning from my Home leave in the middle of 1934 I continued my investigations with the strain 'Tjiwidej', using the sample taken with me to Europe and kept in stab-culture, but without any further precautions. It was the first time since 1930, the year I started my experiments with this strain, that it failed me: the only explanation left was a further dissociation in this strain. I had indeed observed some atypical colonies on the plate, small round colonies without the typical fringe, and two additional series of rats were, therefore, inoculated, one with a vaccine prepared with the apparently unchanged typical variant, the other with that prepared with the atypical variant. Little did I imagine that the typical variant, which showed no morphological alteration whatever, could have entirely lost its antigenic potency. I then appealed to the original strain preserved in stab-culture and uninterruptedly kept in the cold storage at Bandoeng. A comparative experiment (inoculation with 1/50 agar-culture, infection as usual) showed undeniably that the immunizing properties of the original strain had remained unaffected.

TABLE VIII.

Type.	Rat.		GUINEA-PIG.	
	Vaccinated.	Controls.	Vaccinated.	Controls.
1. Tjiw. sm. Europe ..	10 : 0/10	} 10 : 0/10	10 : 6/4	10 : 1/9
Pure typical variant ..	10 : 0/10		..	..
Pure atypical variant	10 : 2/8		..	..
2. Tjiw. sm. Europe ..	10 : 0/10	} 10 : 0/10 {	10 : 7/3	} 10 : 0/10
„ „ Bandoeng ..	10 : 10/0		10 : 10/0	

As soon as the manufacture of the live vaccine, produced to the amount of at least 50,000 doses weekly, was no longer a matter of difficulty, I more closely examined the problem of spontaneous loss of virulence in a number of plague strains from 5 to more than 15 years old and stored at 5°C. in stab-agar-culture after receipt or after isolation here. The strains concerned are those that were used in my differential diagnostic research of the *Pasteurella* group in 1924-1925 in addition to those which had been isolated from different areas of the Preanger in subsequent years. Next to the strains 'Tjiwidej' and 'Java' I experimented upon 12 other strains available from different places: the strains 'Maasland' and 'Sadko' isolated from rat plague on board of ships in Amsterdam in 1920, the strain 'Smyrna' (1918) obtained from Kral's museum, the strain 'Dupré' from the epidemic in Paris (1920), the strain 'Bombay humana' and 'Bombay rat' (1919) from Bombay, the strain 'Harbin' from the second pneumonic plague epidemic in Manchuria (1920-1921), the strain 'Medan' from the small epidemic on the East Coast of Sumatra (1918) and four more strains obtained locally from the Preanger in the period 1925-1929.

As a preliminary experiment all these strains were first sub-cultured in broth and one guinea-pig was inoculated with 1 c.c. of this culture of each strain; if this infecting dose caused no ill effect whatever, a second guinea-pig was injected with a whole agar-culture. If even this dose appeared harmless, the whole stab-culture was cut to pieces each of which was seeded in a broth tube; after sub-cultivation of each of these tubes on agar a full agar-culture of these sub-cultures was each inoculated in a guinea-pig. By this proceeding I succeeded in proving that the strain 'Medan' was still virulent, notwithstanding that the same dose of a full agar-culture derived from the stab-culture in the usual way (after seeding of a loopful in broth) had yielded a negative result. Even more striking was the finding that the original strain 'Tjiwidej', preserved in stab-culture in September 1929 after rat sub-passage and which since January 1930 had yielded absolutely negative results in many animal tests, appeared to be still virulent.

Of the 14 strains investigated in this way, 11 appeared still to be virulent, 8 already after the first inoculation of 1 c.c. of the broth culture, 3 after injection of an entire agar-culture ('Smyrna', 'Medan', 'Tjiwidej', the last two only after exhaustion of the whole stab-culture), regaining their original degree of virulence after some few animal passages. Three strains ('Sadko', 'Dupré' and 'Java', of the last of which the original culture appeared meanwhile to have vanished) had become absolutely avirulent, viz., a dose of a whole agar-culture subcutaneously injected into guinea-pigs caused no symptoms at all (buboes, etc.). Following Kolle and Otto (*loc. cit.*) and Strong (*loc. cit.*), I chose a whole agar-culture as maximum dose, as these authors consider a strain as suitable for human vaccination only if the subcutaneous injection of guinea-pigs at a dose of 2 agar-cultures is not fatal; afterwards Strong (1908*a* and *b*) reduced the dose limit to 1 agar-culture. The condition on which the choice of this dose is based, in my opinion, is insufficient, not so much because of its being arbitrary but especially because death and not the presence of symptoms is considered the criterion of the noxiousness of a vaccine. I myself should not allow a strain to be used for immunizing purposes, which can give rise to buboes in guinea-pigs notwithstanding the fact that a whole agar-culture is not able to kill them, a not exceptional experience. Conversely, I should not be opposed to a strain that is liable, in larger doses, to kill test animals from toxæmia; even a dead vaccine is apt to do so. The Haffkine vaccine in a dose of only $\frac{1}{4}$ c.c. gives a percentage mortality of 20 to 30 in rats from acute intoxication; this was, however, never considered a contra-indication to the vaccination of man with a dose of 3 c.c. to 5 c.c. Each strain, even 'Tjiwidej', retains enough of its toxicity to remain fatal to test animals if not from subcutaneous anyhow from intraperitoneal injection. For convenience' sake, however, I adhered to the dose of 1 agar-culture as a comparative base of qualifying strains avirulent on the condition that buboes were absent in test animals.

Thereafter I investigated the question as to whether avirulent variants could be isolated from those strains, which had retained their virulence. As regards the markedly attenuated strains 'Medan' and 'Smyrna', this seemed rather probable in connection with the behaviour of the strain 'Tjiwidej', but for the other strains this remained an open question. To solve this problem a broth-culture of each of these strains was diluted to such a degree as to obtain well-isolated colonies when one drop was smeared on a blood-agar plate. After 48 to 72 hours

some 10 colonies were sub-cultured in broth and from broth on agar. Finally 10 guinea-pigs were inoculated with a full agar-culture, each originating from one colony and presumably, for the greater part if not all, from one bacillus of the same strain. When a guinea-pig remained unaffected, 10 more guinea-pigs were inoculated each with an entire agar-culture, originating from the same avirulent colony, in order to make sure that a really avirulent variant had indeed been secured. If none of the guinea-pigs survived, the experiment was repeated with 10 other freshly isolated colonies. Finally, the survivors of these experiments were infected in the usual way quickly to obtain an impression of the antigenic value of the strain concerned. The results of these experiments are collected in Table IX:—

TABLE IX.

STRAIN.			Virulence (+ = vir.) (- = avir.)	Infection with 10 different colonies (full agar- culture).	INFECTION.		Infection with test dose.
Number.	Name (Date of isolation).				With 10 other colonies (full agar- culture).	With one same aviru- lent colony (full agar- culture).	
I	Maasland 1920	+	10 : 0/10 ₅₋₈	10 : 0/10 ₅₋₈	..	..	
II	Sadko .. 1920	-	..	..	..	..	
IV	Smyrna .. 1918	+	10 : 9/1 ₁₃	..	10 : 10/0	19 : 3/16	
VI	Dupré .. 1920	-	..	..	..	..	
VII	Bombay humana 1919	+	10 : 1/9 ₅₋₆	..	(10) 9 : 8/1 i.c.	10 : 9/1	
VIII	Bombay rat 1919	+	10 : 7/3 ₄₋₆	..	10 : 10/0	17 : 16/1 i.c.	
X	Harbin .. 1920-1921	+	10 : 0/10 ₃₋₆	10 : 0/10 ₃₋₆	..	..	
XI	Java (1920) 1926	-	..	..	..	..	
XII	Medan .. 1918	+	10 : 10/0	..	10 : 7/3*	17 : 17/0	
XIII	Preanger 1925	+	10 : 0/10 ₄₋₇	10 : 0/10 ₅₋₆	..	..	
XIV	Tasikmalaja 1926	+	9 : 0/9 ₃₋₄	10 : 0/10 ₃₋₄	..	..	
XV	Bandoeng 1929	+	10 : 8/2 ₆₋₈	..	10 : 10/0	18 : 17/1	
XVI	Soemedang 1929	+	10 : 1/9 ₃₋₅	..	10 : 10/0	(11) 10 : 9/1	
XVII	Tjiwidej .. 1929	+	10 : 10/0	..	..	10 : 7/3	
Controls ..			..	..	..	10 : 0/10	

* = toxic death; i.c. = intercurrent death.

The subscript figures indicate the average number of days of survival after infection.

From this it appears that next to the strain 'Tjiwidej' not less than 6 out of 10 virulent strains gave rise to avirulent variants; these strains do not all belong to markedly attenuated strains such as 'Smyrna' and 'Medan', but among them are 4 out of 8 strains whose virulence was apparently intact. Of the last group, in two ('Bombay rat' and 'Bandoeng') the greater part of the bacilli (7 and 8 respectively out of 10 colonies) was avirulent, of both the others, however, only 1/10th (1 out of 10 colonies). The possibility, suggested by the seemingly avirulent parent cultures of the strains 'Tjiwidej' and 'Medan' which appeared actually to have conserved some virulence, by closer investigation was substantially in agreement with Hadley's contention that in many, if not in all, pathogenic bacteria highly virulent and avirulent organisms may be available in the same culture*. This explains the sudden return to virulence of an apparently avirulent culture so often observed, which is actually due to the fact that not all organisms of the culture concerned were avirulent. Only a culture originating from one avirulent bacillus (i.e., colony) is absolutely avirulent and the chance of its return to virulence may be considered as excluded.

This dissociation, now well established, is the more striking since no morphological differences between the colonies can be detected: all the colonies show the typical aspect of a small hemisphere with an irregular fringed zone. In 4 strains ('Maasland', 'Harbin', 'Preanger' and 'Tasikmalaja') no dissociation could be established by this method after repeated experiments; this does not, however, exclude that no dissociation existed, since the examination of hundreds of colonies would be required to prove an absolutely negative result. The experience that this dissociation occurs in some strains within some months and is absent in others for long years, must be accounted for by the individual behaviour of bacteria. Owing to our relatively rough biochemical and serological methods the essential of these intracolonial changes will probably remain inaccessible to us.

According to the results obtained by infection of the survivors (Table IX, last column) the immunizing potency of all but the strain 'Smyrna' was rather satisfactory. Since the vaccinating dose amounted to an entire agar-culture I made a series of experiments in rats and guinea-pigs with all 10 avirulent strains—of which 7 were variants of virulent parent cultures—hoping to obtain an even better antigen than the strain 'Tjiwidej'. A comparative experiment with the strain 'E. V.' from Madagascar, that was kindly sent to me by Dr. Girard, was also made. The vaccination was performed as usual with one injection of 1/100th agar-culture, followed by infection with the usual test dose (in guinea-pigs a ten-fold dose) 3 weeks later. The results of these experiments (4 series) are collected in Table X. As not all strains were used in every series, in the last 2 columns the results of the comparative experiments in rats and guinea-pigs with the strain 'Tjiwidej' are mentioned; these are exactly comparable with those yielded by the strains according to the first two columns.

* Some 9 more strains have since been examined, isolated from virulent plague matter (6 from blood of plague patients, 3 from rat plague) and preserved in stab-culture. In not less than 6 of them I succeeded in ascertaining avirulent variants in the still highly virulent parent culture; the oldest of them had been isolated 3½ years before, the youngest six months only.

TABLE X.

STRAIN.		RAT.		GUINEA-PIG.		STRAIN 'TJIWIDEJ.			
						RAT.		GUINEA-PIG.	
No.	Name.	Results.	Percentage survival.	Results.	Percentage survival.	Results.	Percentage survival.	Results.	Percentage survival.
II	Sadko ..	(10) 10 : 1/9	10	(10) 10 : 0/10	0	10 : 4/6	40	10 : 8/2	80
IV	Smyrna ..	(10) 10 : 1/9	10	(10) 10 : 0/10	0	10 : 4/6	40	10 : 8/2	80
V	Dupré ..	(20) 20 : 10/10	50	(20) 20 : 8/12	40	20 : 13/7	65	20 : 18/2	90
VII	Bombay humana	(30) 30 : 21/9	70	(30) 30 : 30/0	100	30 : 19/11	63	29 : 27/2	93
VIII	Bombay rat	(30) 30 : 22/8	73	(30) 30 : 30/0	100	30 : 22/8	73	30 : 27/3	90
XI	Java ..	(20) 20 : 12/8	60	(20) 20 : 20/0	100	20 : 15/5	75	19 : 19/0	100
XIa	Java (toxic)	(40) 40 : 29/11	72.5	(40*) 36 : 36/0	100	40 : 28/12	70	39 : 36/3	92
XII	Medan ..	(20) 20 : 2/18	10	(20) 20 : 9/11	45	20 : 13/7	65	20 : 18/2	90
XV	Bandoeng ..	(20) 20 : 5/15	25	(20) 20 : 17/3	85	20 : 13/7	65	20 : 18/2	90
XVI	Soemedang	(30) 30 : 15/15	50	(30) 30 : 30/0	100	30 : 22/8	73	30 : 27/3	90
XVIII	Madagascar	(30) 30 : 15/15	50	(30) 29 : 29/0	100	30 : 24/6	80	29 : 28/1	96.5
XVII	Tjiwidej ..	(40) 40 : 28/12	70	(40) 39 : 36/3	92	..	..	..	..
	Controls ..	(40) 40 : 1/39	2.5	(40) 40 : 0/40	0	..	..	..	..

* 4 toxic deaths.

From these figures it will be clear that two of these strains ('Sadko' and 'Smyrna') are absolutely worthless, and two ('Dupré' and 'Medan') possess only slight antigenic potency; the remaining strains have satisfactory immunizing value, though it sometimes markedly differs in rats and guinea-pigs. This is most striking as to the strain 'Bandoeng' with which 25 per cent of the rats survived against 85 of the guinea-pigs; especially in the first series the difference was striking: 70 per cent mortality in rats while the guinea-pigs all survived. In the strains 'Soemedang' and 'Madagascar', yielding exactly the same results, the difference is also marked and it is somewhat less pronounced as regards 'Java'. The strains 'Bombay humana', 'Bombay rat' and 'Tjiwidej' yielded the best results; it must be taken into consideration, however, that the survival rate obtained with the strain 'Tjiwidej' in these 4 series is below that of the average

rate of 80 per cent reached in all experiments with a vaccinating dose of 1/100th culture (cf. Table II); in two of the four series the percentage mortality was not less than 60 and 40 respectively, whereas a mortality rate of 40 was seen only once in the former extensive series of experiments. I, therefore, assume that the antigenic value of the strain 'Tjiwidej' in rats compares favourably with that of both the Bombay strains, though this does not appear from this small number of experiments.

As regards guinea-pigs, the strain 'Tjiwidej' must, however, be put behind them, not so much on account of the slightly differing mortality rates as on account of the incidence of symptoms of illness observed in survivors. While almost all guinea-pigs immunized with the strain 'Tjiwidej' showed distinct, sometimes even large buboes after infection, these were absolutely absent in those immunized with the Bombay strains, just as in those immunized with the strains 'Java', 'Madagascar' and 'Soemedang' and less pronounced in those immunized with the strain 'Bandoeng'. In agreement herewith the temperature of the 'Tjiwidej' guinea-pigs in the first days after infection rises to a somewhat higher level (39°C.) than that of animals inoculated with the other strains compared here, remaining normal (38.5°C.) against 40°C. and higher in controls. Apart from this, the 'Tjiwidej' guinea-pigs seem absolutely healthy and the impression imposes itself that the few failures, as far as they are not due to secondary pneumonic plague, are due to some intercurrent disease, even when post-mortem examination proves the presence of plague bacilli in the spleen.

A rather remarkable variant is the strain 'Java toxic' which, contrary to the strain 'Java', was not isolated from the original strain (which was lost in the meantime) but from its oldest sub-culture available since 1926. This variant, when subcutaneously inoculated, was occasionally fatal to guinea-pigs, not only with large doses but even with a dose of 1/100th agar-culture, post-mortem examination showing the typical aspect of plague though without buboes. In smears of the spleen, as a rule studded with necrotic spots, bacilli are scarce but cultures of spleen matter always give a positive result. When some 10 guinea-pigs are inoculated with such a sub-culture the effect is invariably the same: all with a rare exception, survive and the typical symptoms of acute plague are never observed no matter the number of sub-passages and the dose. Though the symptoms approximate those of death from toxæmia, it is curious that they manifest themselves rarely after 2 days but mainly as late as 5 to 7 days or more after the injection. Besides this variant, even in a dose of a whole culture, does not cause death in rats which are far more susceptible to the toxic action of the plague bacillus. Perhaps this suggests the possibility of the existence of an intermediate form which, though devoid of virulence, has not yet completely become saprophyte. If plague bacilli are apt to multiply for some time in the organism despite gradual destruction, it is conceivable that an increasing amount of toxic products may act upon the most susceptible among guinea-pigs.

Six to eight hours after a subcutaneous inoculation of his avirulent strain Strong (1908a and b) found numerous bacilli still viable at the place of injection, but usually no longer reclaimable in cultures after 24 hours. Strong, therefore, contends that the process of immunization occurs as a true vaccination, the organisms multiplying in the tissues for probably over 100 and more generations

and its successive groups of receptors stimulating the production of the corresponding groups of amboceptors in the animal body. It, therefore, might not be difficult to understand why immunity derived from vaccination in plague is higher than that obtained from the injection of dead organisms. From my experiments with the strain 'Tjiwidej', it appeared that bacilli were present in the tissues by far longer than 24 hours and reclaimable in cultures from the site of injection as well as from the organs especially from the spleen as late as 7 days after a subcutaneous injection. I do not believe, however, that this is due to an active increase of the organisms and that this accounts for the fact that a live vaccine compares favourably with a dead one. This difference, in my opinion, can easily be explained by the antigenic properties being intact in a live vaccine contrary to a dead vaccine artificially killed by heat or by the addition of some disinfectant, both of which are deleterious to them*.

Anyhow, active increase in the animal body of the strain 'Java toxic', concerned here, seemed probable and I, consequently, tried to prove its presence in some of the guinea-pigs dead from an injection of a whole agar-culture (round about 5,000 millions viable bacilli). When bacilli were not too scarce in smears of the spleen, spleen matter was sub-cultured on blood agar plates in order to estimate the number of viable organisms with the dilution method already described. In two cases of death 5 and 11 days after the injection their number amounted to 500 and 1,500 millions respectively; of a third guinea-pig, dead after 7 days, the spleen contained more than 5,000 millions as a proof of increase of the bacilli. As a matter of course the possibility that all organisms injected would have been transported to the spleen and remained there intact for a week may be excluded; besides, the liver, as a rule, shows the same typical aspect with sporadic bacilli in its smears, so that in this organ too rather a large number of bacilli is presumably present.

Apart from this strain 'Java toxic' the above series of experiments shows that there are several avirulent strains of great immunizing potency, characterized, however, by differential results in rats and guinea-pigs. Most of these strains possess an antigen especially active in guinea-pigs but producing less or even slight effect in rats. The strain 'Tjiwidej', on the other hand, is the best antigen for rats, but must be put behind others as regards guinea-pigs. This difference is of a qualitative nature, as it cannot be overcome by increasing the dose.

This striking fact is of great practical significance for the vaccination of man, since a qualitatively different action of various plague antigens may be expected in man too. As it is not feasible to carry out comparative experiments with different antigens in order to establish how man stands as compared with the rat and the

* When the live vaccine is stored at 5°C. as standard suspension of 1/10th agar-culture per c.c., the number of viable bacilli remains unchanged for at least 4 weeks and its antigenic potency is fully maintained. Stored at room temperature (22°C.) the number of viable bacilli quickly decreases in the course of 7 to 14 days, though the immunizing properties remain almost intact. This suggests that spontaneous death of the bacilli is less noxious to the antigen, at least at the beginning, than their artificial death. The preservation of its full activity at a temperature of 5°C. is of great advantage as it allows of the manufacture of the vaccine being independent, to some extent at least, of the consumption. The preparation of the vaccine in its successive stages is now carried out weekly instead of daily (from stab-agar-culture in broth, from broth on a preliminary agar-culture, that—washed off in concentrated suspension—is used for the final cultivation). Though the preparation of a live vaccine is a matter of greater difficulties than that of a dead vaccine, we almost always had a sufficiency of such a stock as to be able to satisfy the demand without interruption.

guinea-pig, vaccination with a mixed vaccine, consisting of 2 or more strains, will guarantee the best results. As soon as amongst the strains which yield a survival rate of 100 per cent in guinea-pigs without any symptoms of illness one will be available, that gives as little reaction in man as the strain 'Tjiwidej', then vaccination will be carried on with a mixed vaccine. Such a vaccine will not present difficulties, its preparation being no matter of greater trouble than that of a single vaccine.

Finally, I would draw attention to the fact that, no matter the theoretical objections that may be advanced against the views on microbic dissociation, so ingeniously developed by Hadley, this phenomenon—when considered from a practical point of view—holds out the prospect of immunization with live vaccines against many epidemic infectious diseases. Just as this research led to a real vaccination against plague, it will prove possible when continuing the work of de Kruif (*loc. cit.*), White (1925), Balteanu (1926), Hadley (1931) and Felix and his collaborators (1934) to prepare absolutely innocuous live vaccines, in the first place against cholera, typhoid and bacillary dysentery, which will be of higher immunizing value than those now in use. As regards veterinary prophylaxis it is obvious that far better results should also be obtainable if this method be adhered to.

CONCLUSIONS.

1. Extensive series of experiments, carried out on wild rats and guinea-pigs with the live, and after some few months, spontaneously attenuated strain 'Tjiwidej', yielded results by far superior to those till now obtained with dead vaccine.

2. In further investigations with a number of strains 5 to 15 years old and preserved in stab-culture at a temperature of 5°C. it appeared that from several still virulent parent cultures avirulent variants could be isolated.

3. This loss of virulence occurs irrespective of the age of the culture and may or may not be associated with alteration in the form of the colony; in the former case the immunizing value, as a rule, is but slight.

4. Even when the typical growth is retained, these avirulent variants show great differences in antigenic potency as proved by animal test: several strains possess highly immunizing value and some appear almost entirely devoid of it.

5. The immunizing properties depend on a compound of antigens of which two at least could be demonstrated; one produces the highest degree of immunity in rats, the other in guinea-pigs. Besides this qualitative difference further quantitative differences obviously exist between these strains as to the partition of these two antigens.

6. From the results of a preliminary vaccination, in accordance with the alternating system, it appeared that mortality from bubonic plague in man can be reduced to 1/10th. The course of the epidemic after mass vaccination of a population of round about two millions also testifies to the value of vaccination as a plague repressive measure of high potency.

7. The use of a mixed vaccine, consisting of two or more strains of different efficacy in rats and guinea-pigs, promises even better results.

8. The presumption is justified that in other acute infectious diseases of an epidemic character the causative agents may also afford absolutely avirulent variants suitable for live vaccines superior to the dead ones in use up to this time.

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THE STERILIZATION OF DRINKING WATER WITH MINIMAL DOSES OF CHLORINE.*

BY

RAO SAHIB T. N. S. RAGHAVACHARI, B.A.,

AND

P. V. SEETHARAMA IYER, M.A.

(From the King Institute of Preventive Medicine, Guindy, Madras.)

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INTRODUCTORY.

THE Great War of 1914 to 1918 advanced the practice of chlorination of water-supplies to a remarkable degree and extension of the process has been so rapid in recent years in all parts of the world that it has come to be regarded as an essential measure in safeguarding water-supplies.

DOSAGE OF CHLORINE.

The amount of chlorine required to kill bacteria in water is very small but considerably larger quantities have to be added owing to the demands made on the chlorine by the oxidizable organic and inorganic matter present in the water. Thus, the maintenance of 0.1 to 0.2 p.p.m. of residual chlorine after a twenty minutes' contact (detectable by the orthotolidine test) is considered to be the standard routine technique of chlorination, in order to ensure continuous action and effective destruction of the pathogenic bacteria. The determination and application of the correct dose is, therefore, of the utmost importance in rendering a water 'safe'. The test for residual chlorine after twenty minutes' contact is likewise considered to be the most accurate method of judging the efficacy of the treatment. The dose of chlorine for any water has, therefore, to be ascertained every day and in some extreme cases more often than once in twenty-four hours. There is, in addition, the question of tastes and odours, which develop in some waters, even when the correct optimum dose is added. In such cases, elaborate methods of de-chlorination

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have to be adopted or the tastes and odours have to be removed by special adsorbing or chemical agents. Finally, one has to contend with the vague fears—freely expressed by many engineers—that chlorinated water is responsible for rapid corrosion of the metal parts of engines and pumps and also of iron distribution mains. In 1926, we had one such complaint from a big municipal pumping plant.

PRELIMINARY EXPERIMENTS.

We, therefore, initiated certain experiments to ascertain if waters could be sterilized by doses of chlorine considerably short of the optimum dose as ascertained by the usual field test. We believed that, if successful, such doses would be acceptable both to the engineers and to the public.

Thirty-two samples including examples derived from wells, rivers, lakes, impounded surface waters, and the effluents from slow and rapid filters were tested. Graduated doses of chlorine, ranging from 0.05 p.p.m. to the actual ascertained optimum dose, were used in each case. The treated samples were then examined for the presence of lactose fermenters. We found that in nearly every case the water showed their absence in 60 c.c. with doses considerably smaller than the optimum. In 5 samples a dose of 0.05 p.p.m. was effective, in 11 cases 0.1 p.p.m. was effective, in 4 others 0.2 p.p.m. was similarly effective and in 10 others 0.3 p.p.m. effected sterilization. The ascertained optimum doses in these cases ranged between 0.5 and 1.5 p.p.m. Although the above results showed that much smaller doses than the ascertained optimum effected sterilization it was not possible to evolve a constant formula or factor that could be made to govern the exact relationship between the minimal dose and the ascertained optimum dose for all waters. As examples, we may give the case of a particular water with a chlorine demand of 1.5 p.p.m. showing the presence of lactose fermenters in 0.01 c.c. which was sterilized by a fifth of that dose, and of another water which required 1.1 p.p.m. by the field test and contained lactose fermenters in 10 c.c. but which was effectively sterilized by 0.05 p.p.m. Under these circumstances, it was not considered desirable to continue the experiments, as the results did not permit the adoption of a constant factor which could be used in the field for arriving at the proper minimal dose from the ascertained optimum dose.

VERDUNIZATION.

Our interest in this subject was, however, revived by the appearance in the *Journal of the American Water-works Association* of two important contributions from Nachtigall and Ali (1934) and Bunau-Varilla (1934) respectively, on the process called 'Verdunization'. In 1916, during the battle of Verdun, Colonel Bunau-Varilla, a French engineer in charge of water-supplies, succeeded in effectively chlorinating the water supplied to the French army with doses of chlorine about a tenth of the determined optimum doses and in producing an absolutely safe water free from any obnoxious taste or smell. This, he claims to have achieved by having recourse to violent agitation of the water immediately after the chlorine was applied in minimal doses. He later advanced the hypothesis that the sterilizing effect was due to the action of chlorine on the organic matter present in the water. It was no longer necessary to add sufficient chlorine to deal with the whole of the organic

matter present in the water. This method of Bunau-Varilla disregards the chlorine demand of the water and stresses the great importance of ensuring 'vigorous agitation' following the addition of chlorine. It is claimed for this method that clear waters from any source can be sterilized with 0.1 p.p.m. and slightly turbid waters from all sources with 0.2 p.p.m. of chlorine, provided vigorous agitation accompanies the addition of the chlorine. Verdunization is now reported to be in successful use in Paris, Lyons, Dieppe and many other cities in France, also in Brussels, Geneva, Lausanne, Genoa, Trieste, Seville and Lisbon, as well as in Algiers, Dakar, Saigon and Caracas.

Nachtigall and Ali (*loc. cit.*) do not, however, concede the above hypothesis of Bunau-Varilla, as in their experience (more or less similar to ours here in 1927), although several samples of water were sterilized with smaller doses than the optimum, there was apparently no uniformity in the relationship between the minimal and the optimum doses. They would, therefore, rely only on the orthodox method of applying the proper optimum doses, ensuring a residual of 0.1 to 0.2 p.p.m. of chlorine.

Bunau-Varilla (*loc. cit.*) criticizes the theoretical considerations relied upon by these two authors and adduces evidence based on what he calls 'three capital experiments' communicated to the French Academy of Sciences. These experiments would go to prove his contention that the 'destruction of the major part of the infectious bacteria is effected by ultra-violet rays emitted by the chemical action resulting from the contact between the organic matter of the water and the particles of chlorine distributed in the whole mass of water by the violent agitation to which it is submitted. The remaining bacteria are destroyed by the direct contact between them and the particles of chlorine which they happen to meet'.

Reiss and Techoueyres (1933) have explained the mechanism of the sterilization of water by 'Verdunization' by showing that when minimal doses of hypochlorite are added to the water there is a definite rise in the oxidation-reduction potential of the water, which is high enough to kill *B. coli* and pathogenic bacteria.

Imbeaux (1933) considers that the sterilizing power of chlorine and chlorine compounds varies with the oxidation potential. Bacterial reduction parallels oxidation potential (in volts), with chlorine, chloramine and azochloramide. Similarly, the addition of ammonia reduces both oxidation potential and sterilizing power of chlorine. Between 0°C. and 25°C. there is a gradual increase in sterilization and in oxidation potential. With increasing turbidity of the water, other factors remaining constant, there is a decrease.

Thresh *et al.* (1933) consider that the nascent oxygen hypothesis of chlorination is no longer tenable and that it is the chlorine that is *directly toxic* to bacteria by forming some compound with their substance and so affecting their physiological processes that life cannot continue. This view would appear to accord with the hypothesis of Bunau-Varilla in some respects.

Race (1918), however, states that though chlorine and its compounds are toxic to bacteria even in minute doses, the smaller concentrations produce merely a transient toxic effect which would permit the reproductive faculty of the organisms to be entirely regained.

Bunker (1935) casually mentions the fact that in one of the municipalities in Spanish America 'Verdunization' has proved a costly failure.

SECOND SET OF EXPERIMENTS.

We applied the Bunau-Varilla method to about sixty samples of water derived from various sources scattered over the Madras Presidency. No conscious selection was made of the samples which arrived in the usual course in the laboratory, and they are, therefore, representative of the typical drinking water sources in actual use. Both clear and turbid waters are included in this number. The technique employed was as follows:—

The theoretical optimum dose was ascertained in each case by the usual field test and the sample was divided into five portions of a litre each. Portion *A* received the optimum ascertained dose and was stirred with a glass-rod a dozen times and allowed to rest for twenty minutes. Portions *B* and *C* each received the minimal dose of 0.1 or 0.2 p.p.m. as the case might be, and *B* was stirred with a glass-rod a dozen times. *C* was taken in a stoppered bottle and vigorously shaken up for ten minutes by lateral movements allowing a foot excursion each time. It was then allowed to rest for ten minutes before being tested along with the others for lactose fermenters. A fourth portion *D* received no chlorine at all but was subjected to the same vigorous agitation in a bottle as *C* above. The object of this was to ascertain what effect the agitation alone, apart from the chlorine, had on the sample of water. Sample *E* was our control, i.e., the water as obtained from the source. All the samples, as detailed above, were inoculated into MacConkey's bile-salt lactose-broth tubes within half to one hour after they had been subjected to the respective treatments noted above. The organisms of the *coli* group present in samples *B* and *C*, which had probably been subjected only to a transient toxic action by the chlorine applied in minimal doses (*cf.* Race, *loc. cit.*), had thus ample opportunities of regaining their vitality in the enrichment medium in which they found themselves in less than an hour of their being acted upon by the chlorine.

Our results, however, show that the toxic action, even of minimal doses of chlorine, has been more permanent than transient. In fact, lactose fermenters were absent in 60 c.c. in all clear water samples in portions *A*, *B* and *C*. The optimum ascertained doses in these cases varied from 0.4 in a few through 0.7 in many to 1.3 in another few. The minimal dose for the clear water samples was a uniform 0.1 p.p.m. In the case of slightly or highly turbid waters, we had only 60 per cent success with 0.2 p.p.m. Some of our samples in this series were definitely discoloured and muddy or opaque. These were drawn from rivers or lakes containing flood water. Our failure to get as good results as in the case of clear waters was probably due to this fact.

The *D* portions of our series, i.e., those which had been subjected to a mere vigorous shaking without any chlorine added thereto, showed that they were bacteriologically very much worse than the control *E* and necessarily still more so than the *C* portions. Bacteria possibly present in the original sample in clumps had apparently got dispersed by the vigorous shaking and owing to this distribution were detectable in the broth tubes even in high dilutions. If this explanation is correct, the hypothesis of Bunau-Varilla, that the ultra-violet rays set up by the

chlorine act instantaneously on the bacteria, would appear to gain some measure of support from the above finding.

We have not been able, from the results obtained in this study, to establish any uniform relationship between the organic content of the waters tested and the ascertained optimum dose of chlorine. In the case of clear waters, however, showing low figures for organic matter (e.g., free ammonia, trace to 0.002; albuminoid ammonia, trace to 0.005; oxygen absorbed by potassium permanganate in 4 hours, Tidy's process, up to 0.040 parts per 100,000), the ascertained optimum dose was nearly always between 0.4 and 0.7 p.p.m. In the case of clear waters showing considerably higher figures for organic matter, this dose varied from 0.4 to 1.5 p.p.m. In the case of hazy and turbid waters, this dose varied similarly. The organic content of a water cannot, therefore, be the sole determining factor in efficient chlorination.

DISCUSSION.

It would, therefore, appear that the bacterial sterilization of drinking waters particularly of the clear waters from wells and filter plants, can be effected by using 0.1 p.p.m. of chlorine empirically. We do not subscribe to the hypothesis of vigorous agitation being the most important factor controlling the success or failure of the minimal dose. For, in the experiments reported above, both our *B* and *C* portions yielded practically identical results. The term 'violent or vigorous agitation' is rather vague, as no means of measuring the vigour or violence has been indicated by the sponsor of the method. As stated above, since both the *B* and *C* portions of our samples have behaved similarly in the enrichment medium, we are left in doubt as to whether our stirring a dozen times with a glass-rod produced sufficient vigour to induce the ultra-violet rays required for the sterilization of the *B* portions. We believe that in actual water-works practice the procedure would be to pass the water and the minimal dose together through a centrifugal pump (McConnel, 1935). In this case the actual contact of each particle with the chlorine *plus* agitation could probably be reckoned in seconds rather than in minutes. On this basis, the mere stirring with a glass-rod a dozen times might be agitation vigorous enough for the bactericidal action in the case of the *B* portions and it is only on that assumption that the Bunau-Varilla hypothesis could be considered to have been established in our experiments. Experiments on a small plant, using minimal doses and a mechanical agitator, are under way and should the results of those experiments point to the same conclusions, this method would doubtless go far to effect considerable economy in the chlorination of water-supplies in India, and would eliminate the troubles met with in the matter of tastes and odours resulting from the application of large doses of chlorine. There would also be no need for a laboratory or trained personnel to control the process of chlorination at each water-works.

CONCLUSIONS.

The results of the application of minimal doses of chlorine to 100 samples of water from different sources scattered over the Madras Presidency, show that *clear* waters from wells, galleries, and filter plants can be sterilized by amounts of chlorine which, in many cases, are from a fifth to a tenth of the optimum ascertained doses.

Should these results of our laboratory experiments be confirmed on a plant scale, the process would result in (i) effecting considerable economies in the chlorination of water-supplies, (ii) ensuring relative freedom from taste and odour troubles and (iii) dispensing with the need for laboratory control of the process from day to day.

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A COMPARATIVE STUDY OF CULTURE AND ANIMAL INOCULATION AS METHODS FOR ISOLATING TUBERCLE BACILLI FROM PATHOLOGICAL MATERIALS.

BY

A. C. UKIL

AND

S. R. GUHA THAKURTA.

*(From the Tuberculosis Inquiry, Indian Research Fund Association, All-
India Institute of Hygiene & Public Health, Calcutta.)*

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INTRODUCTION.

IN the course of our work on the isolation of the tubercle bacillus from materials derived from pulmonary and other forms of tuberculosis (Ukil, 1930, 1933), we had to employ different methods for the purpose, viz., direct culture from uncontaminated materials, culture from contaminated materials after preliminary treatment with chemicals to destroy the secondary organisms, and culture after guinea-pig passage. The isolation of the tubercle bacillus from contaminated materials presents many difficulties. Isolation by animal passage is not only a slow method but is more costly. The price and cost of maintenance of two guinea-pigs, which we utilize for each material, for 8 weeks at Calcutta is approximately Rs. 2-8-0, whereas the cost of 6 tubes of any of the suitable media does not exceed an anna or 1/40th of the cost of the animals. Guinea-pig inoculation has long been widely used as a safe, sensitive and reliable method for isolating tubercle bacilli. While a small amount of material is required for cultures, comparatively large amounts (8 to 10 times) are required for animal inoculation. Culture methods would certainly have several advantages over animal inoculation, if it could be proved that they were as accurate and reliable as guinea-pig inoculation for indicating the presence of tubercle bacilli in a material. Apart from the question of cost, culture takes a shorter time for isolation when it can be utilized for typing even in a small laboratory and the method is free from any chances of vitiating the

findings by intercurrent infections or spontaneous tuberculosis developing in the animals. It would be worth while, therefore, to know how guinea-pig inoculation compared with culture as a method for isolating tubercle bacilli from pathological materials and whether the culture method could be substituted for animal inoculation.

The culture method has been employed recently in several quarters, resulting in the employment of improved media and technique of isolation. Egg has formed an essential constituent of many media devised by Dorset (1902), Lubenau (1907), Petroff (1915*a* and *b*), Loewenstein (1924, 1933), Sumoyoshi (1925), Hohn (1926), Petraghini (1927), Corper and Uyei (1928), and Woolley and Petrik (1931). The presence of egg-yolk has been considered to be more important than that of the white. The use or addition of potato as a favourable component of media has been advocated by Pawlowsky (1888), Corper and Uyei (1928), Calmette and others. Glycerine has been used in many media as a stimulant to the growth of the tubercle bacillus. The growth-promoting influence of certain vegetable extracts, such as vitamin B, starch, etc., has also been studied.

It has been stated (Corper and Uyei, 1928) that several thousand tubercle bacilli per c.c. (100,000 according to some) must be present in a material to enable them to be demonstrated by microscopic examination in smears. Many of them may not be in a living condition, while the presence of secondary organisms renders a preliminary treatment with chemical digestives or with dyes necessary. The preliminary treatment of materials with chemicals, such as antiformin (Uhlenhuth and Xylander, 1908), caustic soda (Petroff, *loc. cit.*) and sulphuric acid (Loewenstein, *loc. cit.*) as well as the use of dyes in the preparation of media, no doubt kills the secondary organisms but has the disadvantage of destroying thinly-walled tubercle bacilli, of retarding the growth of others and of 'denaturing' them. The strength of the solution and the duration of treatment by the acid or alkali are important factors to be considered in this connection. The optimum dilution of the solution and the period of digestion have, therefore, got to be found out. The behaviour of certain strains towards these chemicals has to be considered as well. Petroff (Malkani, 1929) and others have shown that 'S' colonies develop better on alkaline media and 'R' colonies on acid media. In the hope that 'S' colonies could be recovered better by alkaline digestion and 'R' colonies by acid digestion, we employed both these methods of digestion in our culture work in the initial stages. The addition of dyes, such as gentian violet, basic fuchsin, crystal violet, brilliant green and malachite green, etc., also inhibit the growth of tubercle bacilli. Attempts have been made by Loewenstein (1924), Sumoyoshi (*loc. cit.*), Corper and Uyei (1928), Sweaney and Evanoff (1928) and other workers to overcome these drawbacks by improving the culture medium.

METHODS OF CULTURE EMPLOYED.

In culturing tubercle bacilli from pathological materials, the subject has to be considered from three standpoints, namely, the treatment of the material, the culture medium and the inhibiting substance.

Treatment of the material.—In case of materials, such as pus from cold abscess, serous fluid, etc., we first tested them for the presence of secondary organisms

by inoculating broth and agar media and incubating for 24 hours. If no contaminating organisms were noticed on the media the next day, the material was inseminated directly on to the special culture media (for growing T. B.) and also inoculated subcutaneously and intraperitoneally into guinea-pigs.

In case of tissues obtained at autopsy and in case of contaminated materials in general, preliminary digestion had to be resorted to with either alkalis or acids. Knowing that any caustic agent, which is intended for eliminating contaminations, will destroy the viable forms of tubercle bacilli according to the resistance of the coat of the bacilli, the strength of the reagent and the length of exposure, we carried out a series of preliminary experiments with different dilutions of both acids and alkalis and digesting the materials for varying lengths of time. In the case of alkalis, we tried a 7.5 per cent solution of antiformin and a 4 per cent solution of NaOH. Twenty to thirty minutes' exposure to antiformin and half an hour's digestion with 4 per cent NaOH were found to be suitable for successful cultures. For an acid digestion 20 per cent, 10 per cent, and 6 per cent solutions of H_2SO_4 were employed. Half an hour's digestion with 6 per cent H_2SO_4 was found to destroy contaminating micro-organisms, while yielding a positive growth of tubercle bacilli in a majority of cases. It must be mentioned that these dilutions gave the maximum number of positive cultures, but there are some strains and young forms which are very susceptible to chemical treatment. In one case, we obtained a positive culture after only 3 minutes' digestion with 4 per cent caustic soda, while longer exposures proved sterile. The same technique of culture was used after treatment with the different solutions.

In order to find out which of the two methods of digestion, alkali or acid, yielded better results, a comparative experiment was undertaken with positive sputum. After half an hour's digestion with 4 per cent NaOH and 6 per cent H_2SO_4 , they were cultured on Dorset's and Hohn's media. The following table shows the proportion of successful cultures and contamination by each method :—

TABLE I.

Digestive employed.	6 per cent H_2SO_4 .	4 per cent NaOH.
Tubercle bacilli recovered on culture in per cent.	75.0	62.5
Tubes contaminated in per cent	51.5	63.6
Average time required for demonstrable growth.	22 days.	32.4 days.

It appears that sodium hydroxide, in the dilution and the duration of exposure employed, has been more detrimental to the tubercle bacilli than H_2SO_4 . Some workers (e.g., Sweaney and Evanoff, *loc. cit.*) think that, although NaOH is more destructive for tubercle bacilli, it has greater concentrating power than H_2SO_4 . Others (e.g., Loewenstein, Sumoyoshi, Hohn, Corper and Uyei, *loc. cit.*), on the other hand, think that, with the acid method, the growth appears a few days earlier and

a larger percentage of successful cultures is obtained. Our results support the latter view. Since these observations were made, we have employed the acid digestion method only in our subsequent experiments.

Media employed.—After ascertaining the method of digestion, we proceeded to choose the most sensitive medium for culture. With this purpose, a sample of sputum with moderate numbers of tubercle bacilli was digested and inseminated into Hohn's, Corper and Uyei's, Petraghini's, Woolley and Petrik's, and Lubenau's modification of Dorset's medium. Of these, Hohn's and Lubenau-Dorset's media do not contain any dye, Woolley and Petrik's (*loc. cit.*) and Corper and Uyei's contain crystal violet and Loewenstein's and Petraghini's (*loc. cit.*) contain malachite green.

The sputum was mixed with an equal quantity of H_2SO_4 and thoroughly shaken. It was next placed in the incubator at 37°C . for half an hour and then diluted with 10 times the volume of normal saline. After shaking thoroughly, the whole was centrifugalized at high speed for 15 minutes. The supernatant fluid was then decanted off, leaving a thin layer above the sediment. This was then thoroughly mixed up and three loopfuls of the emulsion were spread and well rubbed on the surface of two tubes of each kind of media. The culture tubes were then placed in an incubator at 37°C . and were examined after 24 hours to see if any contamination had taken place. Thereafter they were examined at weekly intervals with reference to the rapidity, amount and character of growth as also to any contaminations that might have taken place. It was also noticed that the media, whose surfaces were rubbed with sterile spatula after a few days of incubation, showed visible growth more easily. As soon as a visible growth was detected, a microscopic examination of smears from surface scraping was done. In case of the digestion of pathological tissues, they were thoroughly ground with pestle and mortar before being mixed with H_2SO_4 . The subsequent treatment was identical with that of the sputum. It was observed that the growth was generally facilitated by rubbing the surface of the inoculated media, with a sterile spatula, a few days after insemination.

Table II illustrates comparative data obtained by culturing sputum on different media :—

TABLE II.
From smear-positive sputum.

Name of medium.	Hohn's.	Corper and Uyei.	Petraghini.	Woolley and Petrik.	Lubenau-Dorset.
Per cent recovered by culture ..	40	54.1	83.3	80	40
Per cent tubes contaminated ..	32	12.5	Nil	Nil	40

It will be seen that Petraghini's and Woolley and Petrik's media gave decidedly better results than the other three media. The following results (Table III), obtained by culturing other pathological materials, such as pus and pleural fluid

TABLE III.
(From pathological materials.)

Materials examined.	Number examined.	Per cent positive in smear.	CORPER AND UYEL.		PETRAONINI.		WOOLLEY AND PETRIK.		LUDENAU'S MODIFIED FORSET.	
			Per cent recovered by culture.	Per cent contaminated.	Per cent recovered by culture.	Per cent contaminated.	Per cent recovered by culture.	Per cent contaminated.	Per cent recovered by culture.	Per cent contaminated.
Pus and pleural fluid.	131	16.7	9.3	9.3	19.0	5.7	16.4	10.6	13.9	18.8
Post-mortem tissues.	185	19.4	5.5	15.5	28.6	12.5	24.2	11.8	19.6	28.3
Both combined	316	18.3	6.7	13.5	24.4	9.5	21.0	11.3	16.8	23.6

and post-mortem tissues, will further prove the superiority of the Petraghini's and Woolley and Petrik's media. The autopsy materials were derived from 14 cases who died of pulmonary tuberculosis, 15 from injury and accidents and the remainder from non-tuberculous diseases. Of the materials in Table III, all the post-mortem tissues and 24 out of the 131 samples of pus and pleural fluid underwent treatment with chemical digestion before culture. Of the 57 positive cultures obtained, 28 strains were recovered from the tissues of cases who died of pulmonary tuberculosis.

It will appear from both these experiments that the Petraghini medium is perhaps the most sensitive of the media studied by us for the growth of the tubercle bacillus from pathological materials. It will be further noticed that the culture method gives more information about the presence of T. B. in pathological materials than in smears obtained by concentration method.

Inhibiting substance.—The purpose of the addition of a dye to the medium is to inhibit the growth of such contaminating micro-organisms as are not destroyed by the previous treatment with digestives and also to facilitate the early detection of the growth of tubercle bacilli as a result of the contrast between the cream colour of the colonies and the coloured background of the medium. Corper and Uyei (*loc. cit.*) tested the action of crystal violet or related dyes, sold under the name of gentian violet, on the growth of tubercle bacilli. They found that at a concentration of 0.01 and 0.002 per cent growth occurred equally well to that in control (without dye) and that crystal violet was slightly more bacteriostatic than gentian violet. In our experiments there was slightly less contamination and slightly more percentage recovery of tubercle bacilli with 0.06 per cent malachite green (Petraghini) than with 0.003 per cent crystal violet (Woolley and Petrik, *loc. cit.*).

CULTURE *versus* ANIMAL INOCULATION IN ISOLATING TUBERCLE BACILLI.

Technique of culture.—In case of *sterile* materials like pus and pleural fluid, the bacilli have been isolated by direct culture of the material on Petraghini and Woolley and Petrik medium in about 23 per cent of cases. Two drops of the material are inseminated in two tubes of each type of medium. Concentration by digestion and centrifugalization kills, as has been said, a certain proportion of the bacilli. In case of *contaminated* materials, they were treated with chemicals as described anteriorly. Three loopfuls (2 mm. diameter) of the centrifugalized sediment were inseminated into two tubes of the above two types of media, incubated and examined from time to time as described above.

Guinea-pig inoculation.—In case of *sterile* materials, like pus or pleural fluid, about one c.c. (or 8 to 10 times the amount used for culture) was inoculated subcutaneously into the right inguinal region of two guinea-pigs and intraperitoneally into a third. In case of *contaminated* materials, 3 to 4 times the amount utilized for culture were introduced subcutaneously into the right inguinal region of two guinea-pigs.

The inoculated guinea-pigs were weighed and examined every week. If a positive culture was already obtained from a particular material or if the animals showed enlarged glands near the site of inoculation or progressive emaciation, they were sacrificed; otherwise every animal was kept under observation for 100 days

before being sacrificed. On autopsy, smears were obtained from the site of inoculation, the contents of the inguinal, pelvic, mesenteric and tracheo-bronchial glands and from the spleen, liver and lungs, in case if any suspicious spots were detected in these organs. The smears were examined by Ziehl-Neelsen stain and carefully examined under the microscope. In case the smear proved positive, a culture was made from the affected tissues.

Results of culture and animal inoculation.—The materials included in Table IV were utilized for both culture and guinea-pig inoculation. Of the 288 materials examined, 44 showed tubercle bacilli in smears under the microscope, 14 of which showed tubercle bacilli only after one of the concentration or homogenization methods was employed. The findings are noted in Table IV :—

TABLE IV.

Nature of materials examined.	Number examined.	NUMBER SHOWING T. B. IN SMEAR.		Totals.
		Untreated.	After homogenization.	
Pus and pleural fluid ..	111	16	2	18
Skin	2	..	..	..
Urine	5	..	..	..
Stool	3	1	..	1
Stomach washing ..	1	..	..	..
Sputum	10	1	1	2
Autopsy tissues ..	156	12	11	23
TOTALS ..	288	30	14	44

Out of 44 smears positive mentioned in the above table, successful cultures were obtained from 32 of them, while in 26 cases inoculated guinea-pigs showed tuberculous lesions.

Among the total number of materials examined, tubercle bacilli could be demonstrated in 102 materials either by culture or by animal inoculation or by both. Of these, 40 were positive by culture alone, 27 by animal inoculation alone and 35 by both the methods. Thus if we take 35 as the common factor, we arrive at the total figures given in Table V. That is, cultures gave us positive information in 75 cases and animal inoculation in 62 cases, or a superiority of 12·8 per cent more information with cultures (taking 102 positive materials into account). Out of 156

autopsy tissues examined, 24 showed giant cells in section, of which 16 showed tubercle bacilli in smears. From these, 17 strains were recovered by culture alone, while 10 showed tuberculous lesions by animal inoculation. Table V will illustrate the position regarding the sensitiveness of the two methods:—

TABLE V.

Nature of materials examined.	Number examined.	Per cent positive in smears.	CULTURE.		ANIMAL INOCULATION.		Number showing giant cells in sections.
			Number positive.	Per cent positive.	Number positive.	Per cent positive.	
Pus and pleural fluid.	111	16.2	32	28.8	30	27.0	..
Skin ..	2	..	..	..	1	50.0	1
Urine ..	5	..	1	20.0	1	20.0	..
Stool ..	3	33.3	1	33.3	1	33.3	..
Stomach washing	1	..	1	100.0	..	..	..
Sputum ..	10	20.0	1	10.0	3	30.0	..
Autopsy tissues	156	14.7	39	25.0	26	16.6	24
TOTALS ..	288	15.2	75	26.0	62	21.5	..

The earliest period at which a culture became positive was 5 days, while the earliest positive finding (demonstration of tubercle bacilli) was obtained from the inguinal gland of an inoculated guinea-pig on the 12th day. The maximum period for a positive finding was the same (100 days) in both. The average period for positive finding was found to be 33.8 days in the case of culture and 48 days in the case of animal inoculation.

DISCUSSION.

It will be noticed that most of our material was derived from non-pulmonary sources and from contaminated material from post-mortem tissues. Although lately the question of culture of tubercle bacilli as a method for diagnosis has attracted attention, yet enough work has not been done with materials from non-pulmonary and autopsy sources. If the material is sterile, the question of a digestive for the destruction of secondary organisms does not arise. A digestive has to be used in the case of septic materials, but, in our efforts to kill or inhibit the growth of secondary organisms in this case, a certain proportion of tubercle bacilli is killed and 'denatured'. In comparing the results of culture and animal inoculation, it should be remembered that a small amount of material is required for culture, while a much larger amount is introduced into the guinea-pig.

For a successful culture, the medium should be carefully selected to produce a sort of selective environment, the material highly concentrated and evenly planted. In spite of all precautions, a certain amount of contamination, due to various causes, is bound to occur. It will be noticed from our findings that the employment of two different kinds of media gives a higher percentage of successful cultures than only one kind of medium. The number of tubes seeded is also important. As has already been stated, the guinea-pig receives 8 to 10 times the material seeded for culture. Hence, for comparable results 8 to 10 tubes (or 4 to 5 if two different kinds of media are employed) should be inoculated, but we used half this number in our experiments. Even, in spite of this, the culture method has given higher results in about one-third of cases, particularly in case of autopsy materials. But, at the same time, animal inoculation has given positive results in a fair number of cases where culture had proved negative. In 46 cases where tubercle bacilli were not found on direct smear examination, the culture gave positive results. Hence, as a diagnostic method it seems to be superior to other methods employed in the hospital.

From the evidence adduced by us it seems that although the culture method is more sensitive than guinea-pig inoculation, it cannot be dispensed with altogether, as a combination of both the methods gives us a higher percentage of positive findings. In addition to the cheapness of cost and ease of technique, the culture method saves considerable time and allows an opportunity to proceed at once with the typing (bovine, human or avian) tests. Of all the culture media we have worked with, the Petragnini medium seems to be superior to others as regards sensitiveness for the growth of tubercle bacilli.

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A STUDY OF NERVE LEPROSY.

BY

E. MUIR,

*Working under the Calcutta School of Tropical Medicine and the Indian
Research Fund Association,*

AND

S. N. CHATTERJI,

Calcutta School of Tropical Medicine.

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THIS paper records the results of the study of eighty-one cases of leprosy. The history and clinical findings were carefully ascertained and a pathological and bacteriological study of the lesions was made in biopsy material.

The object of this study has been to find out the path of infection in nerve leprosy, the nature of the pathological process inside the nerves, and the way in which this pathological process brings about various clinical phenomena.

GENERAL CLINICAL DESCRIPTION OF CASES.

Of 81 cases, 71 were classified under nerve leprosy. Of these, 51 showed only single lesions (N-1) and 21 multiple lesions (N-2). Of the remaining 7, 2 were slight cutaneous (C-1) and 5 were more advanced cutaneous (C-2 or C-3).

We have selected for the most part 'initial' cases, i.e., those in which the disease had not advanced far, though in some the duration was considerable, and it will be noticed that in 53 of them there was only a single observed lesion. In the majority of these there was, in addition to the affected nerve, and connected with it, a definite visible skin lesion with a more or less circumscribed margin. This lesion showed such signs as anæsthesia when touched with a feather, absence of pain when pricked with a pin, keratosis, hypopigmentation, depilation, anhydrosis, erythema, induration and visible or palpable thickening. In some cases erythema,

induration and thickening were uniformly marked throughout the lesion, and in others they were confined to the margin, the centre being comparatively thin, flat and pale. Outside the border of such lesions there was frequently an area of skin showing no visible change, but with anæsthesia to light touch and pain. In a few cases no visible skin lesion was present, but the normal-looking area of skin supplied by an affected sensory nerve branch was anæsthetic.

The length of nerve with palpable involvement (thickening and tenderness) varied in different cases. In some only a small branch was thickened for a distance of a few inches; in others more than one sensory branch supplying the affected skin area was thickened; while in others again one or more mixed nerve trunks, such as the ulnar, showed thickening and tenderness on pressure.

In some cases the thickening of the nerve was uniform, while in others the course of the nerve branch was dotted with a series of small nodules, the inter-nodular nerve lengths being palpable but considerably thinner. This was particularly noticeable in branches of the lateral cutaneous nerve of the forearm. These nodules, when incised or sectioned, were found to contain caseous matter and sometimes white pus.

The small nerve branches, when thickened, seldom gave rise to pain, unless they happened to be situated over a bone, as at the elbow or knee, against which the nerve might be compressed by any hard object striking against it.

When larger mixed nerve trunks were affected, pain was a much more common phenomenon. This was especially frequent in the ulnar nerve; and here in addition to pain in the thickened nerve there was frequently pain in the corresponding hand, this being accompanied by anæsthesia and wasting of the small muscles of the hand and occasionally, through disuse of the hand, of muscles of the forearm. In most of these nerve-trunk cases there was a large or small visible anæsthetic lesion of the skin in the distribution of the ulnar nerve, but the area of anæsthesia extended far beyond the visible lesion.

In most cases with ulnar involvement the nerve showed palpable thickening only in its superficial course, that is below the wrist and above the elbow. But in some cases abnormal tingling could be elicited by striking the forearm over the nerve in the intermediate space, showing that this portion of the nerve was also affected to a certain extent.

BACTERIOLOGICAL FINDINGS.

The 71 neural cases were classified as such partly in virtue of the fact that we failed to find bacilli in the skin but in 51 of them (35 N-1 and 16 N-2) bacilli were found in one or more nerves. Bacilli were found in varying numbers both in the skin and nerves of all the cutaneous cases.

Caseation or abscess formation was found in the nerves of 19 (13 N-1 and 6 N-2) of the neural cases and in 2 of the cutaneous (C-2) cases (in one of these as the result of intravenous mercurochrome injections), and bacilli were seen in connection with all of these with the exception of one.

The following Table gives a list of the nerves examined and the number under each nerve found positive and negative :—

TABLE.

Name of nerve.	Number positive.	Number negative.	Totals.
Ulnar	20	5	25
Radial	8	4	12
Medial anti-brachial ..	10	5	15
Femoral cutaneous ..	0	1	1
Great auricular ..	2	3	5
Sural	2	1	3
Peroneal	9	2	11
Supra-clavicular ..	1	1	2
Infra-patellar ..	2	0	2
Supra-orbital ..	1	0	1
Saphenous ..	1	0	1
Dorsal anti-brachial ..	2	0	2
TOTALS ..	58	22	80

In all the nerves examined, whether they showed acid-fast bacilli or not, considerable cellular granuloma was present.

DESCRIPTION OF SELECTED CASES.

To save space we have chosen 37 typical cases out of the total 81. We have written short descriptions of them and have illustrated them by means of 27 photographs and 24 sketches. For easy reference the figures which appear on the illustrations correspond with the numbers of the cases.

Cases illustrated in Plate I.

Case 12a, abscess of ulnar nerve just proximal to elbow. The capsule of the abscess is formed of the thickened and stretched epineurium. In *Case 12b* the abscess is shown opened. In the base of the cavity is the thickened nerve. The needle indicates the opening through which white pus has emerged from the interior of the nerve and distended the abscess cavity. Above and below the abscess is shown the thickened ulnar nerve and a thickened branch. A few acid-fast bacilli

induration and thickening were uniformly marked throughout the lesion, and in others they were confined to the margin, the centre being comparatively thin, flat and pale. Outside the border of such lesions there was frequently an area of skin showing no visible change, but with anæsthesia to light touch and pain. In a few cases no visible skin lesion was present, but the normal-looking area of skin supplied by an affected sensory nerve branch was anæsthetic.

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Cases illustrated in Plate IV.

Case 5a shows a section (low power) of a small piece of nerve tissue removed from the thickened distal branch of the superficial peroneal nerve in a C-1 N-1 case. *Cases 5b* and *5c* show increasing magnifications of parts of the same section. In the latter, large numbers of bacilli are visible, but comparatively little cellular response. Smears from the skin showed a few acid-fast bacilli in a raised, erythematous and anæsthetic macule. The section was stained by Ziehl-Neelsen and hæmatoxylin. There was only one small skin lesion found on the dorsum of the left foot.

Case 7a is from a section of a nerve in a C-3 case. It is stained by Ziehl-Neelsen and a modification of Bielchowsky's method, so as to show both bacilli and nerve fibres. Note the masses of bacilli without destruction of nerve fibres. *Case 7b* is a higher magnification of part of the same section. Note absence of infection of the nerve sheath in the upper part of the photo.

Case X does not belong to this series of cases. It is a photomicrograph taken from a leprous nerve section stained by modified Bielchowsky's method. The nerve is cut obliquely. In the centre is a granular mass which has destroyed the nerve fibres. At the margin the nerve fibres are seen stained black. The appearance, on moving the field of the microscope and focusing different planes, was that of a stick of asparagus surrounded by horsehairs.

Cases illustrated in Plate V.

Case 3 shows massive infection in a section of a thickened branch of the right sural nerve. This was a N-1 case with an anæsthetic patch on the foot. There was a granulomatous reaction to the bacilli, but no caseation. The only other lesion was a small depigmented patch on the back. Smears from the skin were bacteriologically negative.

Case 4a shows section of branch of dorsal anti-brachial cutaneous nerve. Note caseation of centre of the branch and the persisting nerve fibres at the margin; no bacilli were found in this part. *Case 4b* shows a teased nerve fibre taken from another part of the nerve with acid-fast bacilli but no cellular reaction. The corresponding skin lesion is shown in Plate VI, Case 4.

Case 19 shows teased nerve fibres taken from slightly thickened supra-orbital nerve. The corresponding skin lesion was raised and erythematous, but not anæsthetic to light touch. Cellular response was slight.

Case 78 shows section of capsule of peroneal nerve in a C-2 N-2 case. Note the intracellular position of the bacilli.

Cases illustrated in Plate VI.

Case 1 shows front and back of left upper limb with anæsthetic lesion of hand, raised and erythematous except in the centre which was flat and hypopigmented; abscess of ulnar nerve, after opening of which pain and tingling were less, but anæsthesia as before; thickening of the branch of the lateral cutaneous nerve with two globular swellings, which on removal and section showed caseation without liquefaction. No bacilli were found in skin smears, or in nerve sheath or fibres, but several single acid-fast bacilli were found in the mass of caseous material.

Case 4, scar due to application of corrosive to leprous lesion $1\frac{1}{2}$ years previously; margin spreading at two points where red and raised; thickened branch of lateral cutaneous nerve which on section showed centrally a necrosed area with new fibrous tissue formation. Acid-fast bacilli were found in section in areas bordering on the necrosis and also between teased nerve fibres taken from branch (*see* Plate V, Cases 4a and 4b).

Case 12 shows anæsthetic and hypopigmented patch of hand and abscess of ulnar nerve; inset shows form of abscess opening through which pus emerged, caseous material coating inner side of capsule, branches of medial cutaneous nerve of arm spread out over capsule (*see* Plate I, Cases 12a and 12b).

Case 13 shows a raised, erythematous and anæsthetic patch at the back of the left wrist with thickening of the terminal distal branches of the dorsal cutaneous nerve of the forearm, and an abscess of this nerve above the elbow. A teased preparation from the branch in the neighbourhood of the skin lesion showed no bacilli; a smear from the pus of the abscess showed six bacilli after examining 100 fields. The ulnar nerve was thickened above the elbow; after removal of the nerve sheath tingling of the fingers diminished, but anæsthesia remained the same. No other lesion was found in the body.

Case 15 shows a small patch, raised, erythematous and anæsthetic at the back of the elbow, and thickening of the supplying branch of the medial cutaneous nerve of the forearm. No bacilli were found in skin or nerve, but the latter was highly granulomatous and showed abundant giant cells.

Case 16, raised, erythematous and anæsthetic lesion of right thumb, with swelling of the supplying nerve branch. On removing and teasing a few nerve fibres from the latter, two acid-fast bacilli were found lying on a fibre. The skin showed no bacilli. This was the only lesion.

Case 17, an N-2 case with multiple, raised, erythematous, anæsthetic, lesions scattered over the body. Smears and sections taken from the thickened sural nerve did not show any acid-fast bacilli.

Case 18 showed a small red, raised and anæsthetic patch just above the back of the middle finger, but anæsthesia extended as far as the wrist. A branch of the radial and two branches of the ulnar nerve showed thickening. On examining the latter, some parts were found granulomatous and others not. In the non-granulomatous parts of the nerve bacilli were found singly and in bunches. Smears from the skin showed no bacilli.

Case 21, an N-2 case with multiple anæsthetic patches scattered over the body and palpable thickening of no fewer than 10 nerves. A patch on the back of the left hand was found with thickening of the supplying branches of the ulnar nerve.

Inset a caseous swelling is shown at the bifurcation of a branch of this nerve. A smear from the caseous material showed six bacilli in 250 fields. A teased portion of the nerve showed absence of granuloma, but large numbers of acid-fast bacilli lying between the nerve fibres. After the excision of the swelling and adjacent nerve there was a certain degree of restoration of sensation to the affected skin. Smears from the skin were negative.

Case 22 shows a lesion of the left leg, the only one found in the body, and thickening of an infra-patellar nerve branch. Smears from the skin were negative, but sections of the nerve showed widely scattered masses of bacilli. The nerve sheath showed no bacilli.

Case 27, an N-2 case with numerous small patches, smears from which were negative. The diagram shows a patch on the lateral aspect of the right knee with thickened nerve branches, one of which has a globular swelling (inset). Three bunches of bacilli were found in the caseous material, but none in the cellular part of the swelling.

Case 29 shows a small raised and anæsthetic patch on the back of the right ring finger, thickening of the supplying branch of the ulnar nerve and also of this nerve above the elbow. The skin smear was negative; two bacilli were found on some fibres removed from the nerve branch.

Case 36 shows a leprous lesion of the skin of the back of the right hand; the front of the thumb was also affected; the centre was flat and hypopigmented, but the margin raised and erythematous; the whole area was anæsthetic to light touch. The ulnar nerve above the elbow was thickened and tender. No bacilli were found in the skin or in the scrapings of the nerve capsule or fibres. Removal of the nerve sheath resulted in great reduction of pain both above the elbow and in the hand, but not of anæsthesia.

Case 37 shows a hypopigmented and anæsthetic area on the back of the right hand with thickening and abscess formation of the ulnar nerve above the elbow. The abscess was opened and the thickened part of the nerve decapsulated. No bacilli were found in the skin, nerve capsule or fibres, but the pus from the abscess contained several large bunches of bacilli and many single bacilli.

Case 39 shows a large lesion of the medial side of right arm with hypopigmentation and anæsthesia, thickening of the medial cutaneous nerve of the forearm and its branches. There was also marked thickening of the ulnar nerve above the elbow. Smears from the skin lesion showed no bacilli, but examination of the medial cutaneous nerve showed a large necrosed area with one bunch of acid-fast bacilli outside the necrosed area but no bacilli in sheath. Duration seven years; gradual extension of lesion at times of recurrent fever attacks.

Case 46 shows an anæsthetic area on the back of the right hand with parts of the distal margin raised and erythematous; both radial and ulnar nerves were markedly thickened. Smears from the skin failed to show bacilli. Fibres of the thickened radial nerve were removed, but failed to show bacilli, the nerve showed a highly granulomatous state (see also Plate II, Case 46).

Case 47 shows a small, flat, hypopigmented anæsthetic patch on the right hand. The thickened branch of the medial anti-brachial nerve supplying the part was excised resulting in almost entire restoration of sensation to the part. A smear

taken from the proximal part of the excised nerve showed 4 or 5 bacilli and a smear from the distal part 10 or 12 bacilli. A smear from the skin was negative.

Case 48 shows a patch on the left forearm, raised, erythematous and anæsthetic; also thickening of the supplying branches of the medial and dorsal anti-brachial nerves. The former shows pea-like thickenings and this portion was excised and sectioned. It showed densely granulomatous nerve bundles with small giant cells. In one bundle there was necrosis with fragmentation of nuclei and formation of fine, reticular fibrous tissue. Excision of the nerve branch produced no change in sensation.

Case 50 shows a large anæsthetic lesion of the forearm, flat and hypopigmented in the centre, raised and erythematous at the margin, with marked thickening, pain and tingling of the ulnar nerve. The nerve sheath was excised resulting in reduction of pain and thickening and giving a lighter feeling in the hand and partial restoration of sensation at the elbow. The thickened epineurium with attached muscular septum was sectioned, but showed no acid-fast bacilli. A few nerve fibres were removed and teased out on a slide; they showed two bacilli in 100 fields. Smears of the skin were negative for bacilli.

Case 54 shows a lesion of the entire right forearm marked by slight hypopigmentation and diminished pain sensation, but with anæsthesia to light touch only behind the elbow. The hand was apparently quite normal. Two branches of the medial anti-brachial nerve and a branch of the radial were thickened. One thickened branch of the former nerve was removed for examination. Sections showed cell-infiltration in foci. There were no bacilli found in the highly granulomatous foci or in the sheath, but a few were found in the slightly granulomatous foci and many bacilli were found lying between the nerve fibres in the otherwise normal-looking areas. In the latter the bacilli were chiefly single, but a few small bunches were found. Smears from the skin failed to show bacilli. The above were the only lesions found in the body.

Case 59, patient of 28 years, who gave a history of having contracted syphilis seven years previously, showed a small anæsthetic area on the back of the left hand and very slight thickening of the supplying branch of the radial nerve. There were also two minute suspicious patches, one on the back of the right hand, and one on the right ankle. The thickened nerve branch was excised for examination, followed by partial restoration of sensation in the anæsthetic area. Sections of the nerve showed one distinct acid-fast bacillus lying on a nerve fibre. Skin smears showed no bacilli.

Case 68 shows a raised, red and anæsthetic patch above the back of the right wrist with two small subsidiary lesions. The irregularly thickened medial anti-brachial nerve branch was dissected out and is represented in the inset. There was a similar lesion with thickened nerve branch on the dorsum of the left foot and a lesion on the left cheek. These lesions were not stained for bacilli.

Case 81 shows a flat hypopigmented, anæsthetic lesion of the back of the right forearm, with a thickened branch of the dorsal anti-brachial cutaneous nerve. At the point where this branch divides was an abscess. The abscess was evacuated and showed bunches of acid-fast bacilli. The smaller terminal branch was excised and sectioned, but no bacilli were found. Smears from the skin were negative for bacilli. No other lesion was found.



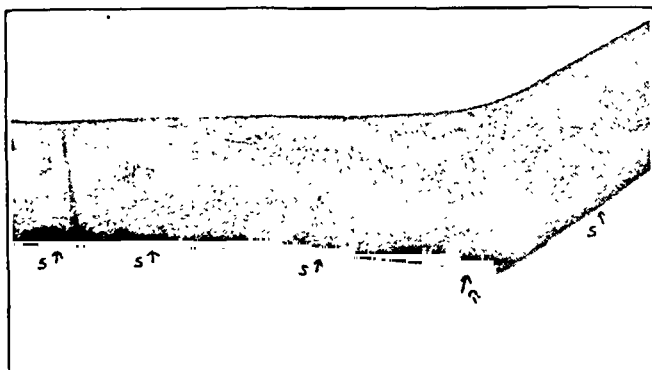
Case 12a.



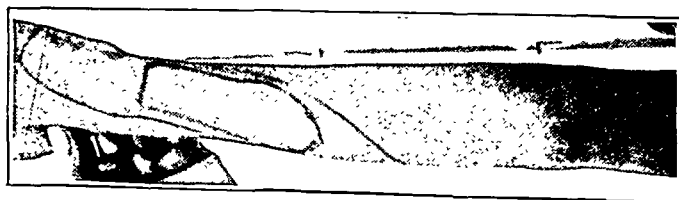
Case 12b.



Case 9.



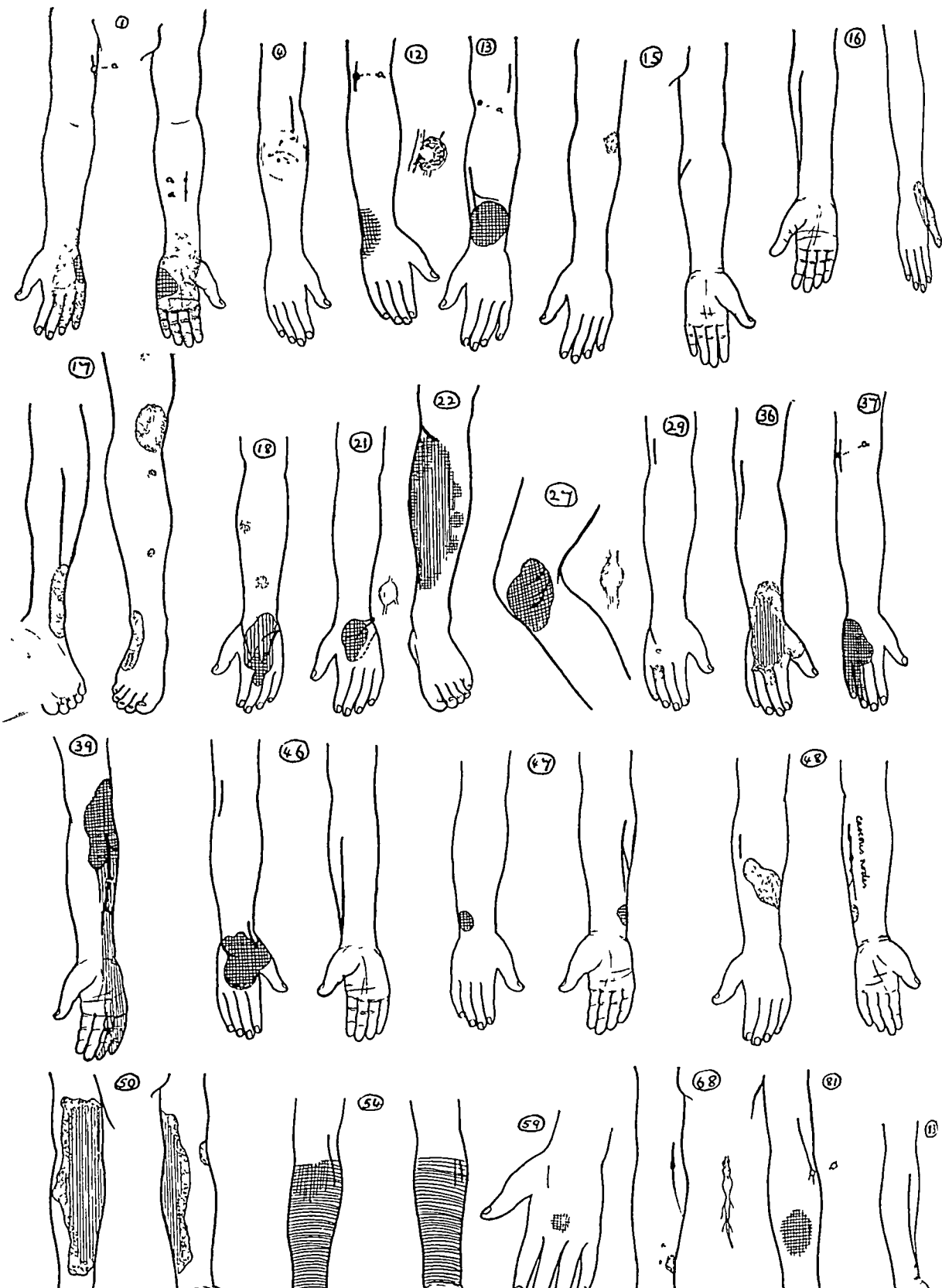
Case 8.



Case 63.

PLATE VI.

Note.—Case numbers are indicated by encircled figures.



Case 83. See also Plate II. Cases 83a and 83b, under which this raised, red, anæsthetic lesion with the thickened branch of the radial nerve is described.

DISCUSSION.

Entry of bacilli and their passage up nerve bundles.

There is considerable evidence to show that *M. lepræ* enter the nerve bundles by way of the skin, passing up sensory nerve branches from the subpapillary and subcutaneous nerve plexus. It is frequently seen that the only nerves affected are those which supply sensory branches to a skin lesion, this being the only lesion present in the body. This is well illustrated in Plate VI, Cases 1, 13, 18, 48 and 54. It is obvious in these cases that infection could not have entered the nerves direct through their blood supply or otherwise than by passing up from the skin.

Infection may spread up a nerve from the skin causing little or no palpable or visible change in the nerve, until it reaches a point of obstruction at which it causes thickening and tenderness (Plate VI, Cases 1, 13, 36, 37, 46 and 81).

Often, however, there may be thickening of a local nerve branch, with little or no apparent skin lesion connected with it, as is seen in Plate VI, Cases 15 and 83, and Plate II, Cases 10 and 83. In other cases, a large mixed nerve such as the ulnar above the elbow, is found thickened, without any lesion of the skin area which it supplies but, as is shown later, there is reason to believe that in at least the majority of such cases entry of bacilli has been through the nerve endings from the skin.

When we section the corium, we find nerves and blood vessels running in close proximity to each other in the subpapillary and subcutaneous plexuses, and round the hair follicles and coil glands. This same proximity is found in the small neurovascular bundles in the subcutaneous tissue. It is in close connection with nerves and vessels in these plexuses and round these epithelial extensions that *M. lepræ* are found in sections of recently infected skin. The organisms lie extra- and intracellularly, and excite varying degrees of cellular response, especially from the endothelial cells of the capillaries.

It appears that the bundles of peripheral sensory and mixed nerves form a specially favourable medium for the multiplication of *M. lepræ*, considerably more favourable than either the skin or the connective tissue sheaths of these bundles (perineurium and epineurium). This is shown in several of our cases, and especially in those illustrated in Plate VI, Cases 4, 16, 22, 39, 50 and 54, Case 4 also being illustrated in Plate V. In these cases there were large and conspicuous leprosy lesions of the skin. No bacilli were found in smears made from the skin or from the perineurium, but bacilli were present (sometimes in large masses) lying between the nerve fibres.

It should be noted here that while the skin was examined, except in a few cases, only by means of smears of material scraped with a sharp knife from the corium, the nerves were examined by scraping and teasing the nerve fibres, and in some cases by sectioning. The more thorough examination of the nerves does not, however, at all account for the greater number of bacilli found in the nerves, as a careful study of the case records will show.

Concentration of bacilli in nerves at certain points.

Under favourable circumstances *M. lepræ* gradually pass up the nerve bundles from the skin, and are found lying between the medullary sheaths of the nerve fibres. They do not penetrate the medullary sheaths. They are probably propelled up the course of the bundles by the lymph flow, and multiply as they pass up. They tend to accumulate at certain points where they meet with obstruction. This obstruction is caused by bending or branching of the nerve, by its constriction under fibrous bands, or by the nerve passing superficially over bony surfaces where it is liable to injury. The results of such accumulations of *M. lepræ* are mentioned later.

One of the commonest sites for accumulation of *M. lepræ* is the stretch of the ulnar nerve between the elbow and the middle of the arm. At the former point it passes between the medial epicondyle of the humerus and the olecranon, and is bound down by a more or less dense and narrow arch of deep fascia; at the latter point it passes behind the intermuscular septum. At both of these points, and especially at the former, the nerve tends to be constricted; and the intermediate stretch of nerve is liable to injury. The constant bending of the nerve also favours the obstruction of the passage of bacilli up the nerve. Hence this is the commonest site of nerve lesions, as is shown by reference to Plate VI, Cases 1, 12, 29, 36, 37 and 46. Another reason for the frequency of affection of the ulnar nerve is the large skin area with which it is connected, and the peculiar liability to injury of that skin surface. Thus the ulnar nerve has through its cutaneous terminals a peculiarly wide and favourable catchment area for *M. lepræ*.

Similarly, the superficial peroneal, great auricular and supra-orbital nerves are liable to injury, and *M. lepræ* in passing up them are subject to obstruction; hence these nerves, next to the ulnar, are among those most commonly affected.

Cellular response to bacilli.

We have mentioned above that the bundles of peripheral nerves form a more favourable medium for the multiplication of *M. lepræ* than do the skin and the connective tissue sheaths of these bundles. Hence *M. lepræ* may be said to be 'neurotropic', applying this term in a special sense, quite different from that generally applied to certain viruses. What this neurotropism is due to it is difficult to say. Attempts have been made to culture *M. lepræ* on nerve fibres, but no better success has yet been attained with this than with other media.

Our present study, however, tends to show that one of the important factors which permit the passage of *M. lepræ* up the nerves and their multiplication between the nerve fibres is the partial or complete absence of cellular response to the organisms as compared with the much greater response in the skin and in the neural connective tissue. This is well illustrated in the cases referred to at the end of the Section on the entry of bacilli and their passage up nerve bundles but especial reference may be made to Case 22 (Plate VI) in which, in spite of an intense degree of cellular granuloma in the skin, along with negative bacteriological findings in both skin and nerve sheath, there were masses of bacilli found in the small supplying nerve branch. Cases 5 and 7 (Plate IV) and Case 82 (Plate III) also show the intense bacillary infection that may be present in the nerve bundles with comparatively

little cellular response. As is mentioned in Case 54 (see Plate VI) there were no bacilli found in the skin or nerve sheath, there were no bacilli found in the highly granulomatous foci in the nerve, a few bacilli were found in the slightly granulomatous nerve areas, but many bacilli were seen lying between the nerve fibres in the otherwise normal-looking areas.

Source of cellular response in nerve bundles.

In the skin the principal cells responding to the presence of *M. lepræ* are the endothelial cells of the capillaries. As the bacilli in the skin lie in close connection with the capillaries and their cells, the latter respond easily to their contact. The same holds good in the vascular connective tissue surrounding the nerve bundles, as is shown in Plate V, Case 78, and Plate II, Case 83; in the latter the cellular proliferation belongs to the perineurium, in which no bacilli are seen; the bacilli are lying on a few adherent nerve fibres.

In the nerve bundles, on the other hand, the vascular capillaries lie centrally, and the bacilli are more or less isolated from the endothelial cells of those capillaries by the medullated fibres. Although the bacilli are apparently more or less uniformly distributed throughout the bundle to begin with, it is at the centre that response first takes place causing granulomatous accumulation and bacillary ingestion and phagocytosis. This spreads outward displacing, blocking and destroying the central nerve fibres till the core of the bundle becomes a granulomatous mass with nerve fibres remaining only as an outside covering. The appearance of such a nerve bundle on longitudinal section, when stained with a special nerve stain, may be compared to a stock of asparagus covered with black horsehairs (see Plate IV, Case X). Such sections will often show an absence of bacilli at the centre, where they have been destroyed by phagocytosis, and their presence at the periphery to which the phagocytic cells have not yet reached. This is well shown in Plate III, illustrating Case 82. In Case 7, illustrated in Plate IV, practically no cellular response has taken place, and massive infection is seen between the nerve fibres; while the perineurium surrounding the bundle is practically free from bacilli. Also in Case 5, illustrated in the same plate, an intermediate stage is shown; a mild degree of cellular reaction has taken place, some bacilli having been ingested by the cells while others lie extracellularly.

Results of varying degrees of cellular response in the skin.

We have stated above that cellular response to lepra bacilli is, in the same subject, stronger in the skin and in the neural connective tissue than in the nerve bundles. The degree of cell response also varies in individuals, being stronger in some and weaker in others. It also is liable to vary in each individual from time to time. It may be taken, however, that any cause which results in an increase or diminution in one tissue will proportionally affect the other tissues concerned. The degree of cell response is in direct proportion to the resistance of the patient to *M. lepræ*.

For simplicity we shall first describe the cytological picture as seen in the corium. As is mentioned above, the cell most concerned in the reaction to *M. lepræ* is the endothelial cell of the capillary. Whether all endothelial cells

respond equally, or whether, as has been suggested by some writers, especial cells of the reticulo-endothelial system are chiefly concerned, it is difficult to say.

The primary and simplest reaction results in the ingestion of the bacilli by the cell. If the response is mild, the bacilli multiply in the cytoplasm of the cell, swelling it out until the typical 'foamy' or 'lepra' cell is formed. At the same time local multiplication of cells takes place, and these in turn ingest bacilli set free by the bursting of lepra cells or created by intercellular multiplication. Along with this a mucoid substance, 'gleea', is formed which cements the adjacent lepra cells and organisms into large bacillary masses.

In more resistant cases cellular multiplication is more rapid, and the cells are packed together more densely. At the same time, bacillary ingestion is followed not by multiplication of the bacilli in the cytoplasm, but by their phagocytosis and destruction. Thus, a totally different histological picture is given from that first described. Instead of the foamy bacillus-laden lepra cell, we have healthy so-called epithelioid cells, tightly packed together into cords, with a clear-cut margin surrounding the neuro-vascular plexuses, hair follicles and sweat glands. Few or no bacilli are found present in these cords.

In cases showing high resistance, the presence of a stronger cellular reaction results in the formation of giant cells, which is the indication of a still more active attempt on the part of the endothelial cells to isolate and destroy the bacilli.

Results of varying degrees of cellular response in the nerve bundles.

In the nerve bundles the reaction of cells to lepra bacilli is similar in nature to, but less in degree than, that in the corium. Also, as we have mentioned above, the bacilli are more isolated from the endothelial cells lining the capillaries in the centre of the nerve bundle. This is because of their distribution over the surfaces of the medullated fibres. For these two reasons, even in cases with high resistance, bacilli may go on multiplying for a time without phagocytosis taking place, and may form themselves into bundles and masses especially at points where there is (as mentioned above) obstruction to their upward movement in the neural lymph stream. When, however, they are at last reached by the proliferating endothelial cells, not only does phagocytosis and giant-cell formation take place in the region where the bacilli are more sparsely distributed, but also necrosis, caseation and even pus-formation result in the regions of bacillary accumulation. This may be followed by masses of bacilli being cut off from the phagocytic action of the cells; they are isolated by the caseous or purulent matter in which they are embedded and may retain their morphological features and acid-fast staining for years. This process is illustrated in Cases 1, 12, 13, 27, 37 and 81, shown in Plate VI, in Plate I, Case 12, and in Plate II, Case 10. In all of these we failed to find bacilli except where they were preserved in the caseous or purulent material.

Caseation and abscess-formation appear to result from the combination of two factors: a considerable accumulation of bacilli at one point, and high resistance of the patient to *M. lepræ* and consequently powerful cellular response.

In our experience, caseation or abscess-formation of this nature seldom occur in the skin, the reason probably being that in resistant cases phagocytosis and destruction of bacilli take place there more promptly, and thus large accumulations of bacilli are unable to take place.

We have, however, seen the sudden skin reaction which occurs in resistant cases of the neural type result in severe ulcer-formation in some three or four cases (not of this series) and Dr. Lowe has drawn our attention to the analogy between this and neural abscess-formation. This confirms the evidence that the skin and nerve lesions in these cases are essentially of the same nature, being however as a rule much more severe in nerve tissue for the reasons mentioned above.

We should mention here that abscess-formation of a somewhat different type does take place not infrequently in the skin in leprosy. In the condition commonly known as 'lepra reaction' or 'lepra fever' large cutaneous or subcutaneous nodules, containing masses of bacilli, break down and form pus, but this pus is, like that of the septic abscess, chiefly composed of polymorphonuclear and other blood cells; there are also masses of bacilli both free and contained in the cytoplasm of these cells. Whereas the pus of the neural abscess is chiefly white and homogeneous, containing few, if any, cellular elements and resembling the cold abscess of tuberculosis.

Causes of varying degrees of cell response.

We have mentioned above that local cell response is increased by the concentration of the bacilli which call it forth, a large mass of bacilli calling forth a greater local effort on the part of the tissue cells than single scattered organisms. Apart from this, there are three factors connected with varying degrees of resistance to *M. lepræ* and of cell response to the organisms in their neighbourhood. These are: the specific resistance of the patient's tissues to *M. lepræ*, the general health of the patient, and what is apparently a form of allergic reaction to *M. lepræ*. Leaving aside in the meantime the third of these three factors, we shall in the first place consider the first two, both separately and in their relationship to each other.

Specific resistance or immunity to M. lepræ.

This is shown by the degree of response elicited in certain cells of the body by the presence of *M. lepræ* in their neighbourhood. As leprosy is for practical purposes chiefly a disease of the skin, subcutaneous tissue and peripheral nerves, we shall study the reactions produced by lepra bacilli in these tissues of the body.

While blood cells also take part, especially if the reaction is strong, it will simplify matters if we confine our attention to the principal cells concerned, viz., the endothelial cells of the capillaries.

The degree of cell reaction to *M. lepræ* varies enormously in different subjects, and this variation is best made clear by contrasting a C-3 case with a typical N-1 case. Let us take for instance Case 7 (Plate IV) and compare it with Case 12 (Plates I and VI). In Case 7 we see the massive infection of the nerve, with little or no cellular reaction. In Case 12 the intense cellular reaction has led to the formation of a large nerve abscess, in which only a few bacilli could be found in the pus and none in the capsule; also the skin lesion is limited to a small patch on the ulnar side of the wrist in which no bacilli were found. In Case 7 the lesion had been noticed by the patient for only eight months. The disease had probably existed for a very much longer time, and massive infection was present in the skin all over the body. But the absence of marked reaction of the tissues, and the almost symbiotic relationship between the cells and bacilli, accounted for the absence of signs which

would attract the attention of the patient. In Case 12 the history gave a duration of three years, but as the cellular reaction to the comparatively few bacilli was intense, the signs and symptoms were marked from the beginning. It is worthy of mention that in the case of skin leprosy there was intense infection of the nerves, although neural swelling and nerve symptoms were comparatively slight; whereas in the case of neural leprosy the infection was very slight. In other words the most marked signs of leprosy are due more to the intensity of the cellular reaction to the bacilli than to the number of bacilli in the body.

We have shown elsewhere (Muir and Chatterji, 1934) that, as in tuberculosis, small infections, especially in adults, tend to raise the resistance; whereas, after a certain point, the increase of bacilli in the body lowers the resistance and the power of the cells to react to, phagocytose and destroy the bacilli. Moreover, the specific resistance of the body to *M. lepræ*, as well as to other acid-fast organisms, is especially low in the first few years of life. We cannot, however, discuss this question here at length, and our readers are referred to the article just quoted.

The general health of the patient.

The importance of the general health in influencing the genesis and course of many infectious diseases has become more widely recognized in recent years. The less toxic the causal organism of a disease, the more important does this factor become. Of the disease-causing organisms of major importance perhaps none is less toxic than *M. lepræ*. Uncomplicated leprosy, even in a marked C-3 case, does not of itself necessarily impair the general health to any marked extent, and therefore complicating factors which interfere with the general health are of extreme importance in influencing the genesis and course of the disease. Any passing condition which depresses the general health (be it a complicating disease, dietary error, or other factor), lowers temporarily the resistance of the body to leprosy infection, so that the bacilli tend to multiply in the body. Even in patients who, as the result of small leprosy infections, have acquired an enhanced specific resistance to leprosy, the temporary impairment of health will for the time being diminish or cancel that specific resistance. If health is restored in time there often follows, due to restored specific resistance, a reaction in lesions within which bacilli have multiplied during the temporary depression. Hence, the formation of the more markedly raised and erythematous skin lesions, especially those of the 'tuberculoid' type with epithelioid and giant cells. Hence also the violent cellular reactions in nerves, resulting in extreme cases in caseation and abscess-formation.

If, however, the depressing factor is too great or lasts too long, the bacilli multiply to such an extent that when health is restored the former raised specific resistance has become replaced by depressed specific resistance; in consequence the bacilli go on multiplying and the disease continues to increase.

Summary of causes of caseation and abscess-formation in nerves.

The following factors combine to cause this condition:—

- (a) Infection with *M. lepræ* of a strictly limited nature.
- (b) High specific resistance to *M. lepræ*. It is difficult to say to what extent this may be inherited; but, as we have shown elsewhere, there

is little doubt that small repeated infections with *M. lepræ* increase, at least for a time, specific resistance to *M. lepræ*.

- (c) Temporary depression or eclipse of this high specific resistance due to some passing impairment of general health. The depressing factor must not be sufficient, however, either in time or duration to allow of a very great and generalized increase of infection; otherwise the high specific immunity becomes changed into permanent low specific immunity to *M. lepræ*.
- (d) This temporary depression of health removes or impairs for the time being the power of the endothelial cells to react to the presence of, and to phagocytose bacilli. In consequence the bacilli multiply in the skin and pass up into the sensory nerve bundles and through them into the mixed peripheral nerves. In some cases the skin retains sufficient reacting power to prevent increase of organisms in its tissues, and the bacilli multiply and spread in neural tissue alone, this being more favourable than the skin for the growth of *M. lepræ*. At points of obstruction the bacilli tend to accumulate in larger or smaller masses.
- (e) Recovery from the temporary depression of health is accompanied by restoration of high specific resistance. The bacilli, which have in the meantime increased in number in the nerves, call forth a vigorous response, the 'recovery reaction', from the endothelial cells of the nerve bundle capillaries in their neighbourhood. The reaction of these cells to scattered bacilli results in the formation of a granuloma composed chiefly of epithelioid cells. Where small bunches of bacilli are present there may be giant-cell formation. Where large bunches or masses of bacilli are present there may be caseation or abscess-formation.

Case 18 (Plate VI) illustrates part of this process. Two years previously the patient had an attack of fever with rigor, probably malaria. Five months ago he first noticed the lesion on the back of his left hand. Sections of the thickened ulnar nerve branch in the neighbourhood of the skin lesion showed bacilli singly and in masses in the less granulomatous or non-granulomatous parts of the nerve, and absence of bacilli where the cellular reaction was marked. The 'leprolin' test showed that the specific resistance of the patient was only moderate in degree, hence the only moderate degree of cellular response and the absence of caseation and abscess-formation.

In Case 10, a much higher degree of specific immunity was present; but this had been temporarily lowered by an attack of kala-azar. The latter disease had been cured three months previously; and restored general health was followed by swelling of the sural nerve and the formation of a marked nerve abscess in the course of that nerve. Bacilli had disappeared, as far as our observations showed, both from the skin and from between the nerve fibres; but acid-fast bacilli were found singly and in masses, embedded in the pus of the abscess.

Several of our other cases show similarly the results of different degrees of cellular reaction; the results varying according to the specific resistance, the duration

and degree of the temporary depressing factor, and the amount and nature of neural infection that took place during the period of depression.

The form of nerve caseation and abscess.—When only caseation has taken place abscess generally lies more or less centrally in the nerve forming a fusiform or pea-like swelling. When pus-formation has occurred the abscess may either be central or may lie outside the nerve as a sessile sac.

In one case described by one of us elsewhere (Muir, 1924) there were three large fusiform abscesses in the course of the ulnar nerve between the axilla and the elbow. On incising the most distal of these, a large amount of white pus was evacuated; and pressure on the other two abscesses evacuated them through the incision. It was found that the stretches of nerve between the abscesses consisted of a hollow tube.

Case 12 (Plates I and VI) illustrates the lateral sessile type of abscess, pus having escaped from the centre of the nerve and ballooned out the thickened capsule.

For the sake of simplicity we have taken the nerve bundle as the unit in describing the process of abscess-formation. When, however, several parallel bundles of a composite nerve are similarly affected, caseous or purulent units will coalesce to form a large abscess. Its thick, fibrous walls may be coated with caseous material, the pus being located in the centre, as in Case 12.

The degree of impairment of nerve function varies with the amount of nerve-fibre destruction and with the degree of pressure exerted by the abscess on the functioning part of the nerve. In Case 12 there was but little loss of function, as the pus had escaped early from the centre of the nerve and exerted very little pressure on the functioning nerve fibres. In others, such as Cases 9 and 63 (Plate I), a rigid thickened capsule prevented expansion of the nerve and there was considerable pressure, and consequently anæsthesia. Excision of the nerve sheath was at once followed by restoration of more than half the lost sensation.

Nerves as a reservoir for M. lepræ.—We have shown above that in the nerves *M. lepræ* are more protected from the phagocytic action of cells than they are in the skin. We have also suggested that this may, at least in part, be due to their sheltered position between the medullated nerve fibres at a distance from the endothelial cells of the capillaries. We have also referred to the fact that, while small infections with *M. lepræ* increase the specific resistance, large infections lower the resistance, or rather produce specific tolerance to this organism. The massive infection in C-2 and C-3 cases, with but little proportionate cellular reaction, noticeable in the skin but even more marked in the nerves, and the negative results with the leprolin test in these cases, give strong evidence of the truth of this view.

There is, therefore, considerable likelihood that the peripheral nerves form the principal reservoir for *M. lepræ* in the body, at least in incipient cases of leprosy. Between the nerve fibres they can multiply, and, in cases with little or even moderate specific or general resistance, they escape any marked control by the phagocytic action of the cells. In the neural tissue they may thus multiply until they reach a number sufficient to produce specific tolerance. Thereafter, the phagocytic action of the cutaneous cells is lowered, and multiplication and spread of the infection becomes possible in the skin. Thus initial neural infection becomes transformed into cutaneous leprosy.

It is difficult to do more than surmise as to how the bacilli pass from one nerve to another. It would seem that escape from a nerve must take place by way of the skin, connective tissue or lymph nodes. We have made careful sections of lymph nodes in many cases of initial and limited nerve leprosy and have uniformly failed to find either bacilli, or signs of cellular reaction which would indicate their having been present. As we have seen above, the cells of both the skin and neural connective tissue react more readily to the presence of bacilli than do those of the nerve bundles.

When, therefore, there is adequate resistance, the bacilli tend to be sealed up inside the infected nerve by the surrounding connective tissue and the distal skin. In evidence of this is the remarkable fact that in the great majority of our cases the disease is confined to the one or two nerves originally infected from the skin lesion in their sensory distribution. While in some cases a marked neural infection has existed, it may be for years, in the one or two nerves involved, the rest of the body has escaped. When, however, resistance is lowered sufficiently to break the seal and make the skin filter pervious, the infection finds its way out through the perineural connective tissue, and other nerves are liable to become infected.

An illustration of the way in which infection may spread through the subcutaneous plexus in the periphery of a leprosy lesion of the skin was shown in some of our sections. As the lesion extended, papules appeared at a short distance beyond the margin. Sections through the margin including a papule and the intermediate healthy skin showed the spread of infection in the subcutaneous plexus passing beyond the original lesion and spreading up the hair follicles to the subpapillary plexus and the papillæ. From there it spread directly through the superficial layers of the skin. Thus the ultramarginal papule became continuous with the advancing lesion. As nerves and vessels are closely mixed together in the perifollicular region, it was impossible to state that the infection passed up to the surface in neural as opposed to vascular tissue, but in the subcutaneous layer the infection was distinctly intraneural. The infection might be compared to a plant like the sweet potato, spreading underground and sending up its shoots to the surface at short intervals.

In Case 4 (Plates V and VI) we have an example of a lesion at first apparently healed and then beginning to spread at two points on the margin of the apparently healed lesion. Sections of the thickened supplying nerve branch showed caseation due to a former *recovery reaction*, but bacilli were found among the nerve fibres outside the caseous area. Apparently the *recovery reaction* had not been sufficient entirely to destroy the bacilli in the nerves, and a subsequent depression of general health allowed the bacilli once more to pass down from the nerve bundles and invade the skin.

In Case 54 we have a somewhat similar condition. The former extensive lesion covering the whole forearm has apparently healed. In the highly granulomatous nerve branches also the bacilli have been eradicated. The skin lesion showed signs, however, of gradual proximal spread, and bacilli were found between the nerve fibres in the non-granulomatous areas of the bundles. This case suggests re-infection of the skin from the nerve branches during a period of depressed general health.

Local and distal symptoms of neural origin.—We have referred above to the non-toxic nature of leprosy and the fact that even in a C-3 case the general health may be excellent. The most distressing symptoms are connected more with neural than with cutaneous leprosy. We have already mentioned the different forms of caseation and nerve abscess and shown that pain, tenderness, anæsthesia and other symptoms are due to pressure caused by cell proliferation, caseous material and pus, and that these symptoms are most severe in cases with very marked central inflammation accompanied by a rigid fibrous capsule which prevents expansion, the fibres being pressed between the swelling interior and the rigid exterior. We have shown, especially by reference to Cases 9 and 63 (Plate I), the effects of removal of the nerve sheath in such cases in reducing anæsthesia, pain and tenderness; and the relief of pain, etc., after opening nerve abscesses.

We would call attention also to the partial restoration of sensation after excision of portions of granulomatous nerve branches in Cases 21, 47, 59 and 83 (Plates II and VI).

Anæsthesia, as found in nerve leprosy, may be divided into two types: primary and secondary. Primary anæsthesia is due to the destruction of small nerve branches in the skin and subcutaneous tissue. Secondary anæsthesia is due to the infection passing up into the larger sensory and mixed nerves and causing pressure upon bundles of fibres which supply healthy skin. Removal of the nerve sheath and the opening of nerve abscesses cause restoration of the latter and not of the former. The diminution of anæsthesia after excision of small diseased branches may be due to the surrounding healthy nerve branches taking up the function of the excised diseased branches. The restoration is too rapid to be explained by growth of axicylinders from the proximal ends of the excised nerves.

Allergic reaction.—*Lepra fever*, otherwise known as *lepra reaction*, is a condition in which there is sudden swelling up of leprosy lesions and appearance of new lesions along with febrile and other general disturbances. It is found chiefly in fairly advanced and progressive cases of the cutaneous type, and generally occurs in patients with poor general health, or in those who are suffering from other accompanying diseases. It may be of a continuous or intermittent type. The sudden and repeated swelling up and subsiding of lesions is not accompanied by a proportionate increase and diminution of *M. lepræ* in these lesions.

There is good reason, in our opinion, for applying the term 'allergic' to this latter form of reaction. Whereas the 'recovery reaction' described above does appear to be as a rule of an allergic nature; it is not a hypersensitization, but a restoration of the temporarily depressed power of the cells to react to *M. lepræ*. While typical cases of each type of reaction are easily distinguished from one another, there are some cases in which the one form may appear to merge into the other.

As mentioned above, in severe reactions of the 'lepra fever' type, cutaneous nodules may break down and give rise to acute abscesses full of pus cells and bacilli, the cells being chiefly phagocytic blood cells. There may also be sudden, painful swelling up of infected nerves. In the case of the ulnar nerve there may be severe permanent impairment of the small muscles of the hand unless pressure is relieved by decapsulation without delay.

Case 8 (Plate I) is a remarkable example of small localized abscesses in a C-2 N-2 case. An abscess of the medial cutaneous nerve is shown, and four scars of previous abscesses are also visible. We find it difficult to classify this case, as the lesions are similar to those found in *recovery reaction*, while the widespread skin infection and the prolonged febrile symptoms point to the *lepra fever* type.

We must confess, however, that little is yet known of the nature of lepra fever; and terms like 'allergy', which have been applied in the study of diseases of a much more toxic nature, should be used with caution in describing leprosy.

SUMMARY.

1. Bacilli enter and pass up nerve branches from the neuro-vascular plexuses of the skin. This may, or may not, be accompanied by clinical skin lesions. Either the local branches, or the distant main nerves, or both, may show clinical changes. Bacilli lie in close connection with neuro-vascular plexuses in the skin and subcutaneous tissue. The nerve bundles form a specially favourable medium for the growth of *M. lepræ*.

2. Bacilli are carried up the nerves from the skin by the lymph flow. They tend to accumulate at points of obstruction.

3. The 'neurotropism' of *M. lepræ* is discussed. This is shown to be at least partly connected with the comparative absence of cellular response to the bacilli in the nerves.

4. The endothelial cells of the capillaries take the most important part in the response to *M. lepræ* in both the skin and the nerves. In the latter the bacilli are isolated from the endothelial cells of the centrally placed capillary by the medullated nerve fibres, and thus may escape phagocytic destruction. In the skin the bacilli lie in close proximity to the endothelial cells, and are thus more liable to be phagocytosed.

5. The effects of varying degrees of cellular reaction to lepra bacilli in the skin and in the nerves are discussed, especially with regard to the formation of nerve abscess.

6. Apart from the degree of local concentration of bacilli, there are three factors which account for varying degrees of resistance and cellular response to *M. lepræ*, viz., specific resistance, general health and allergic reaction.

7. The course of events is traced in the formation of caseation and nerve abscess; the forms which nerve abscesses assume are described and discussed in relation to their effect on nerve functions.

8. The nerves form an important reservoir for bacilli where they may multiply until sufficient tolerance is produced to overcome the greater resistance in the skin. This is followed by spreading of the bacilli to other nerves, and frequently by generalized infection.

9. Pain and other symptoms are relieved in suitable cases by excision of the nerve sheath of thickened nerves, opening of abscesses and excision of diseased nerve branches. A distinction is made between primary and secondary anæsthesia.

10. 'Recovery reaction' is distinguished from allergic reaction.

and epidemic form. Only four cases of typhus were admitted to hospital during 1935 from among the British troops in the area, two from Solan, one from Dagshai and one from Kasauli. A clinical account of these cases, and also of two Indian cases treated at the Cantonment General Hospital at Kasauli, has been given by Bush (1936).

It has been the experience of workers in other countries that in order to establish typhus strains from human sources the most favourable time for taking the patient's blood is within the first three or four days from the first onset of fever, i.e., before it is possible to obtain a positive Weil-Felix reaction, or to make a clinical diagnosis with certainty. Arrangements were, therefore, made to take blood from any patient with fever admitted to hospital in Kasauli as soon as a preliminary test for malaria parasites had proved negative. Unfortunately, all the cases of fever where blood was taken early in the disease subsequently proved not to be typhus, and on only three occasions was blood obtained from genuine typhus cases before the fever had subsided. In each case the Weil-Felix reaction given by the patient's serum was positive with *Proteus* OXK and negative with OX19 and OX2*. An account of the attempts made to establish typhus strains from these three cases is given below. No opportunity arose for obtaining blood from a human case whose serum agglutinated OX19 :—

(1) Case GK was a British soldier. The highest Weil-Felix reaction recorded was 1 : 200 with OXK on the 22nd day of the disease. Blood taken on the 11th day was defibrinated, and inoculated intraperitoneally into two guinea-pigs, GK/1 and GK/2, each of which received about 5 c.c. GK/1 developed a temperature of 103.0°F. on the fourth day, and was found dying on the fifth. Sub-passage was made with spleen emulsion into guinea-pigs GK/3 and GK/4, both of which died within 24 hours. GK/2 did not develop fever, but lost weight rapidly from the fourth day, and was found in a dying condition on the eighth. Sub-passage was made with spleen emulsion intraperitoneally into guinea-pigs GK/5 and GK/6, but both were found dead next morning, and the strain was lost. Post-mortem examination of these animals showed no special lesions except congestion of the spleen, and in one case hæmorrhagic foci in the lungs. Cultures of the blood were not taken, but the symptoms noted were similar to those which occurred in some of the guinea-pigs used in sub-passaging strains RD and WL, and which were proved to be due to infections with *B. ærtrycke*. It is considered probable that the deaths of the six guinea-pigs used in attempting to establish strain GK were also due to secondary infections with this organism.

(2) Case RD was an Indian of the poorer class, who had been employed as a cook. The highest Weil-Felix reaction recorded was 1 : 5,000 with OXK on the 11th day of the disease. This patient developed broncho-pneumonia, and died on the 22nd day from the first onset of fever. Blood taken on the 9th day was defibrinated, and inoculated intraperitoneally into two guinea-pigs. Each of these animals developed a moderate febrile reaction, commencing on the 9th and 10th days after infection respectively. The strain was carried on through four generations by intraperitoneal inoculation of spleen or brain emulsion, one or more animals in each sub-passage developing a mild febrile reaction, which commenced about the

* All Weil-Felix tests referred to in this paper were carried out with concentrated alcoholized suspensions of *Proteus* X strains.

8th to 10th day. None of the three animals inoculated at the fifth sub-passage showed any reaction, and the strain was lost. None of the passage animals showed any outward sign of a scrotal reaction, although in some cases thickening and injection of the tunica vaginalis with increased exudate was noted post mortem. Organisms resembling *Rickettsia* were seen in scanty numbers in scrapings from the tunica vaginalis of certain of the passage animals. Two of the 19 guinea-pigs used for sub-passaging this strain died within the first few days following inoculation, with symptoms similar to those noted above in the case of strain GK. A culture taken from the heart-blood of one of these resulted in a pure growth of *B. ærtrycke*.

Histological examination of the brain of one of the guinea-pigs of the third sub-passage revealed the presence of a number of lesions resembling those described by other workers as typical of infection with typhus. This animal had developed a febrile reaction on the 11th and 12th days after infection, and again on the 15th to 19th days, and was killed on the 21st day. Sections of the brain showed proliferative lesions associated with the smaller blood vessels. Various stages were seen, varying from a concentration of one or two layers of neuroglia cells round a capillary to larger infiltrations resulting in the formation of a nodule, with more or less complete obliteration of capillaries. Small perivascular hæmorrhages were also present. The lesions, which were most numerous in the cerebral cortex, appeared to resemble very closely those described by Lewthwaite (1936) in his account of the histological changes met with in the brain in tropical (rural) typhus.

(3) Case WL was a British soldier. The highest Weil-Felix reaction recorded was only 1 : 50 with OXK, on the 22nd day of the disease, but clinically the case was considered to be typical of typhus. Blood taken on the 10th day was defibrinated, and inoculated intraperitoneally into two guinea-pigs. One of these developed a mild febrile reaction on the 8th day, and the strain was maintained by sub-passage of spleen or brain emulsion intraperitoneally in guinea-pigs through 7 generations, one or more animals in each sub-passage developing a febrile reaction which commenced about the 8th to 10th day, as in the case of strain RD. The fever was of a mild type, seldom exceeding 103·0°F. Most of the reacting animals were killed for sub-passage, and the full course of the pyrexia was observed in six cases only. In these, the initial fever lasted only two or three days, but in four of the cases there were irregular rises of temperature for short periods during the second and third weeks after inoculation. In the majority of cases there was no loss of weight, but a few animals lost about 10 per cent of their body-weight during the initial three days of fever. At the eighth sub-passage, none of the four animals inoculated showed any reaction, and the strain was lost. A definite scrotal reaction was observed in one guinea-pig at the fourth sub-passage, though it was less marked than that regularly noted in guinea-pigs inoculated with strains recovered from wild rats. As in the case of strain RD, organisms resembling *Rickettsia* were occasionally seen in scanty numbers in scrapings from the tunica vaginalis of passage guinea-pigs and white rats. During the course of these experiments, 16 guinea-pigs out of 31 used developed high fever and died during the first few days after inoculation. Cultures taken from the heart-blood of three of these animals yielded a pure growth of *B. ærtrycke* in each case, and it is considered probable that the deaths of all these animals are attributable to secondary infection with this organism.

Attempts were made to establish strains RD and WL in rabbits by inoculating passage virus into the anterior chamber of the eye, by the method practised successfully by Nagayo and his colleagues (1931) in Japan in the case of tsutsugamushi disease, and by Lewthwaite and Savor (1932) in Malaya in the case of rural typhus. An irido-cyclitis was produced in several animals, but attempts to carry on the strain in further passages were unsuccessful, except in one instance. Four rabbits had been inoculated intra-ocularly with spleen emulsion from a reacting guinea-pig of the fifth sub-passage of strain WL. One rabbit developed an irido-cyclitis, and the strain was carried on through four generations by intra-ocular inoculation of aqueous humour. Only about 20 per cent of the inoculated animals showed any reaction, however, and the strain was lost at the fifth intra-ocular sub-passage, three months after the primary inoculation of the patient's blood into guinea-pigs. Scrapings from Descemet's membrane were examined in the case of several of the reacting rabbits, but in spite of careful search, *Rickettsiæ* were not observed.

On two occasions passage virus from strain WL was inoculated into monkeys [*Silenus (Macacus) rhesus*]. In the first experiment two monkeys, WL/1 and WL/2, each received an emulsion containing half the brain of a passage guinea-pig of the fourth generation subcutaneously. Monkey WL/1 developed a temperature of 103·4°F. on the fifth day, and the fever continued until the 10th day, after which it did not exceed 102·4°F. The highest temperature recorded was 104·9°F. on the 9th day. Monkey WL/2 developed a temperature of 103·0°F. on the 7th day, and the fever continued until the 11th day, after which the temperature did not exceed 102·4°F. The highest temperature recorded was 103·3°F. on the 9th day. The reaction produced in these two animals resembles that which followed the inoculation of one of our rat strains into a monkey, which showed a rise of temperature on the 8th, 9th and 10th days after infection (Covell, 1936b).

In the second experiment, three monkeys were inoculated subcutaneously with material derived from a passage guinea-pig of the 5th generation. Monkey WL/3 received an emulsion containing one-third of the spleen, monkey WL/4 received 4 c.c. of defibrinated heart-blood, and monkey WL/5 received an emulsion containing one-half of the brain. The first two animals showed no rise of temperature during an observation period of three weeks, but monkey WL/5 developed a temperature of 103·0°F. and 104·0°F. on the 15th and 16th days after inoculation respectively. None of the animals used in either experiment showed any definite symptoms of illness, and in no case was the result of the Weil-Felix test significant, the highest agglutination titre recorded being 1 : 70 with OXK.

The Weil-Felix test was carried out with the sera of a number of rabbits which had been inoculated either intraperitoneally or intra-ocularly with passage material from strains RD or WL. Out of 29 rabbits on which the test was performed at five-day intervals over a period of 40 days, 6 gave some degree of agglutination with OXK (Table I).

Though the agglutination titres were low, the waxing and waning curves are considered to be suggestive. The results given by the serum of WL/28 are interesting as showing the late development of agglutinins for *Proteus* strains in the rabbit, to which Felix (1933) has drawn attention. None of the sera gave any significant degree of agglutination with OX19 or OX2.

Eighteen white rats were inoculated intraperitoneally with passage material from strains RD or WL. Organisms resembling *Rickettsia* were observed in scrapings made from the tunica vaginalis of some of these animals, but in scanty numbers only. The temperatures of the rats were not recorded. The sera of 12 of the animals were tested for the Weil-Felix reaction, and agglutination was observed in two cases with OX19 in a dilution of 1 : 50, a finding which will be referred to later. There was no significant result with OXK.

TABLE I.

Results of Weil-Felix tests in rabbits with OXK.

Days after inoculation.	NUMBER OF RABBIT.					
	RD/4.	RD/6.	WL/2.	WL/7.	WL/20.	WL/28.
0	0	0	25	0	0	25
10	0	25	125	0	0	0
15	0	45	125	17	50	0
20	25	25	70	15	27*	0
25	50	0	60	25	..	0
30	35	0	0	15	..	55
35	17	0	..	0	..	70
40	15	0	..	0	..	85

* WL/20 was killed for passage on the 21st day after inoculation.

As regards cross-immunity experiments, 10 guinea-pigs previously inoculated with passage virus from one or other of the human strains all reacted in a typical manner when inoculated with one or other of the rat strains. On the other hand, out of 8 guinea-pigs which had previously reacted to infection with one or other of the rat strains, only one showed a febrile reaction following inoculation with a human strain. These experiments were carried out, however, when the human strains were apparently weakening, and when only a small proportion of normal guinea-pigs were showing a febrile reaction to them. It is considered that such evidence as is available indicate that there is little if any cross-immunity between the human and rat strains.

DISCUSSION.

The results of the experiments detailed above show that the clinical and serological reactions produced in laboratory animals by our rat strains differ markedly in certain respects from those which follow the inoculation of strains derived from human cases of typhus.

The rat strains produce a severe febrile reaction in guinea-pigs accompanied regularly by an acute specific orchitis, and typical intracellular *Rickettsia* are demonstrable in abundance in scrapings from the tunica vaginalis. Two strains have been maintained in these animals for 9 months in the laboratory without difficulty. In white rats, though there is no outward sign of a scrotal reaction, typical intracellular *Rickettsia* are found in large numbers in scrapings from the tunica vaginalis of infected animals. The sera of white rats and rabbits infected with the rat strains have yielded a markedly positive Weil-Felix reaction with *Proteus* OX19 in a considerable proportion of cases.

With the human strains, on the other hand, a febrile reaction in passage guinea-pigs occurred less frequently than with the rat strains, and, where present, it was of mild degree only. A scrotal reaction was of rare occurrence, and in no case has a reaction been observed which was comparable in severity with that regularly produced by the rat strains. It has not been found possible to maintain human strains in guinea-pigs beyond the 4th sub-passage in one case and the 7th in another. Organisms resembling *Rickettsia* have been found in scanty numbers only in scrapings from the tunica vaginalis of passage guinea-pigs, and these were not sufficiently typical to warrant a certain diagnosis in the absence of other evidence. In white rats, the same remark regarding *Rickettsia*-like organisms applies; whilst in both white rats and rabbits the response to the Weil-Felix test, if present at all, was observed in comparatively low dilution only.

The results of our attempts to establish strains of typhus from human sources, though disappointing, were not altogether unexpected, in view of the experiences of other workers.

Shortt and D'Silva (1936) have described experiments carried out with material obtained from human cases of typhus in Kasauli in 1934. As in the experiments detailed above, the sera of all the cases had given positive Weil-Felix reactions with *Proteus* OXK only, and the blood was in no case taken in the very early stages of the disease. Evidence of a definite infective process after inoculation of laboratory animals was limited to those which received direct intraperitoneal inoculation of patients' blood. A mild febrile reaction was noted in only two out of 28 guinea-pigs used, and in no case was a scrotal reaction observed. Six out of 8 white rats inoculated with patients' blood reacted with fever, and organisms resembling *Rickettsia* were seen in scrapings from the tunica vaginalis of guinea-pigs and white rats. One rabbit out of three gave a positive Weil-Felix reaction with *Proteus* OXK. The serological results in the case of the white rats were of interest, in view of the findings recorded by Anigstein (1933) with the sera of passage rats (*vide infra*). Out of 8 sera tested, 5 gave some degree of agglutination with OX19, the highest titres being 1 : 125 in one case, and 1 : 50 in two others. It has been our experience that whilst the sera of normal white rats very frequently show standard or total agglutination with OXK in a dilution of 1 : 50, it is rare for any agglutination to occur with OX19, even in a dilution of 1 : 25.

In Malaya, similar difficulty has been experienced in establishing strains from human sources. Fletcher and Lesslar (1925) inoculated a number of guinea-pigs with the blood of typhus patients, and two animals reacted with fever, but the strain was lost after the second sub-passage. Lewthwaite (1930) inoculated 113 guinea-pigs with the blood of rural typhus patients or with material from passage

animals, but only 7 developed fever, and in no case was a scrotal reaction observed. Attempts to maintain strains beyond the third sub-passage in guinea-pigs and rats failed. In rats, 25 developed a febrile reaction out of 87 inoculated from 62 patients.

Anigstein (*loc. cit.*), also working in Malaya, obtained a febrile response in 11 per cent out of 440 guinea-pigs inoculated with virus obtained directly or indirectly from human cases of typhus. In the case of one strain, which was derived from a fatal case of rural typhus and which was maintained for 11 generations, a scrotal reaction was observed in 5 guinea-pigs out of 67 used; in experiments with another strain, this reaction was noted in only one case out of 27. In inoculated rats, 35 per cent gave a positive Weil-Felix reaction, 20 per cent reacted with fever, and 12 per cent showed some degree of scrotal reaction. Anigstein notes that the Weil-Felix reaction in rabbits was inconstant, and of a lower titre than that recorded in rats. It is interesting to note that the sera of two rats inoculated with passage virus agglutinated suspensions of *Proteus* OX19, despite the fact that the original virus belonged to a K strain, as has been noted above in the case of certain white rats used in Shortt and D'Silva's experiments and in our own. Additional significance is given to these findings by the result of an experiment of Anigstein, in which a transformation of the XK to the X19 form apparently occurred in the rat, and was transmitted in this form through the guinea-pig to man. It is noteworthy also that Lewthwaite and Savor (1932) have established a typhus strain from wild rats which produces agglutinins sometimes for OX19 and sometimes for OXK.

In only one instance in Malaya have the numerous attempts made to establish a strain of rural typhus (i.e., K form) in guinea-pigs or rats from human sources succeeded, in spite of the relatively abundant clinical material available. This strain was derived from blood drawn from a patient early in the disease and inoculated into guinea-pigs which had been fed on a vitamin-deficient diet (Lewthwaite and Savor, 1933). Passage guinea-pigs have reacted with fever, but never with scrotal swelling, and the Weil-Felix reaction in rabbits inoculated with passage virus has on various occasions been positive with OXK, but never with OX19. A strain of urban typhus (i.e., agglutinating *Proteus* OX19) has also been successfully established in guinea-pigs by these authors. This strain produces a scrotal reaction in the majority of passage guinea-pigs.

It was on account of the difficulties encountered in maintaining strains of rural typhus in guinea-pigs and rats that Lewthwaite and Savor (1932) attempted to establish strains in rabbits by the intra-ocular method of Nagayo. These attempts were successful, and it has been found possible to initiate and maintain strains of rural typhus without difficulty, after the first two or three sub-passages have been successfully negotiated. Our own failure to establish strains of typhus in rabbits from human sources by the intra-ocular method has been a great disappointment. It must be remembered, however, that an opportunity has not yet occurred to obtain blood for this purpose direct from a patient in the early stages of the disease, and it is felt that this method offers the best prospect of success in future experiments.

NOTE ON THE STAINING OF *Rickettsiæ* IN SMEARS.

It is generally agreed that the best method of demonstrating *Rickettsiæ* in smears is by the use of Giemsa's stain, but various modifications in technique have

been suggested. The writer has obtained satisfactory results in the case of scrapings from the tunica vaginalis of rats and guinea-pigs by the following method: The smear, after thorough drying but without preliminary fixation, is treated with Giemsa's stain made up in the proportion of 20 drops of stain to 15 c.c. of distilled water for 4 hours. The stain is flushed off with distilled water, and the slide allowed to dry. It is then rinsed in xylol and afterwards passed rapidly in succession through absolute alcohol, 90 per cent alcohol, 50 per cent alcohol and 15 per cent alcohol. It is finally washed in distilled water, and allowed to dry.

NOTE ON THE TECHNIQUE OF THE WEIL-FELIX TEST.

During the past few months, 1,260 sera have been examined by the Weil-Felix test with concentrated alcoholized suspensions of both *Proteus* OX19 and *Proteus* OXK, and in a number of cases with *Proteus* OX2 also. In carrying out these tests, much time and labour have been saved by adopting the technique advocated by Bridges (1935). In this method the Dreyer's tubes (after the addition of serum) are filled rapidly to approximately half an inch from the brim with normal saline, instead of measuring the latter by drops from a pipette. As Bridges has shown, the error thus introduced is so slight as to be negligible. We have, however, slightly modified the procedure as follows: Before the tubes are taken into use, 24 drops of saline are dropped into each from a Dreyer's pipette, and a mark is made with a diamond at the upper level of the fluid. In performing the test, saline is poured into each tube up to the mark (after the addition of serum), before the single drop of concentrated suspension of *Proteus* X is added.

ACKNOWLEDGMENTS.

I wish to express my thanks to Dr. R. Lewthwaite, who in a personal communication to the writer very kindly described in detail the technique of his intra-ocular operation; to Lieut.-Colonel R. F. Bridges, R.A.M.C., Officer-in-Charge, Enteric Laboratory, Kasauli, for supplying the concentrated alcoholized suspensions of *Proteus* X strains used in the Weil-Felix tests, and for examining a number of cultures from passage guinea-pigs; to Captain F. K. Bush, R.A.M.C., for allowing me access to the patients under his charge at the British Military Hospital and Cantonment General Hospital, Kasauli; and to Sub-Assistant Surgeon B. N. Lahiri, I.M.D., of the Pasteur Institute of India, for his help in carrying out numerous animal passages.

SUMMARY.

An account is given of attempts to establish typhus strains from human sources in laboratory animals.

Two strains were maintained in guinea-pigs for four and seven generations respectively, and the latter was further carried on for four sub-passages in rabbits by the intra-ocular method, after which it was lost. The various reactions produced by the strains in laboratory animals are described.

Although the attempts to establish typhus strains from human sources ended in failure, they are recorded because it is thought that an account of the experiments carried out may be of assistance to other workers in India.

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THE RÔLE OF MALARIA IN THE CAUSATION OF CIRRHOSIS OF THE LIVER.

BY

RAO BAHADUR T. S. TIRUMURTI, B.A., M.B., C.M., D.T.M. & H. (Lond.),

AND

M. V. RADHAKRISHNA RAO, M.B., B.S., PH.D.

(From the Department of Pathology, Medical College, Vizagapatam.)

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In a previous communication (Tirumurti and Radhakrishna Rao, 1935) we pointed out that the common association of malaria and cirrhosis of the liver in India has given rise to divergence of opinion regarding the rôle of the former in the causation of the latter. This problem is an important one in India on account of the frequency with which malaria is associated with cirrhosis of the liver (Hughes and Shrivastava, 1927 ; Hughes, 1933 ; Hameed, 1933 ; Radhakrishna Rao, 1933) and the ease with which the latter could be eradicated in time, if the former could be proved to be connected with the latter as cause and effect, by early detection and a more thorough treatment of malaria.

We have already pointed out (Tirumurti and Radhakrishna Rao, *loc. cit.*) that in our experience malaria *per se* is not a direct cause of cirrhosis of the liver. But on account of the importance of the problem and the divergent views regarding the same, we reinvestigated the whole question by improved staining methods and the results form the basis of this communication.

MATERIAL AND METHODS OF STUDY.

As a correct understanding of the problem under discussion depends on an accurate study of the nature and genesis of fibrosis, if any, in chronic malarial livers, we bestowed our attention on this line of investigation. In addition to our own collection of chronic malarial livers in the Pathology Museum of the Vizagapatam Medical College, we also investigated the autopsy material (chronic malarial livers) obtained from the Madras Medical College and the Stanley Medical School, Madras,

through the courtesy of Dr. A. Vasudevan and Dr. T. Bhaskara Menon respectively. Briefly our method of investigation was as follows :—

From each of the specimens of chronic malarial livers several thin pieces were taken so as to include in the parenchyma the different orders of the divisions of the portal and hepatic venous trees, to study the changes, if any, at the different levels of the vascular and biliary trees. The pieces of the liver were fixed in 10 per cent neutral formalin. In the case of old museum specimens, the pieces were first washed in running tap-water in a histological washing tank for 24 to 48 hours and then fixed in 10 per cent neutral formalin. For routine examination paraffin sections were stained with Ehrlich's acid hæmatoxylin and eosin : for staining the connective tissue, the paraffin sections were stained with Weigert's iron-hæmatoxylin and van Gieson stain. Frozen sections were stained with the Foot and Ménard's (1927) silver carbonate impregnation method to bring out the reticulum of the liver. It may be mentioned here that this method of staining the reticulum of the liver has given very satisfactory results in our hands.

REPORT OF INVESTIGATION.

The macroscopical and microscopical appearances in the liver in the material under investigation could be understood from the brief descriptions of some of the typical cases given below :—

Case I (Plate VII).—The liver weighed 40 oz.; surface smooth; dark brownish in colour; dark brownish on section; lobular markings distinct; the organ cuts with resistance; consistency firmer than normal; Prussian-blue reaction positive.

Microscopically—no loss of lobular pattern; sporadic fatty change; the sinusoids contain Kupffer cells loaded with malarial pigment; no fibrosis; endo-phlebitis of a collecting vein.

Case II.—The liver weighed 40 oz.; normal in size; surface slightly uneven; capsule thickened in places; section dry and of a deep brownish colour; cuts with resistance and very firm; Prussian-blue reaction positive.

Microscopically—slight thickening and irregularity of the capsule; slight condensation of the reticulum around the central veins; no loss of lobular pattern; sporadic fatty change; sinusoids prominent and empty; Kupffer cells loaded with malarial pigment; slight widening of the portal spaces. Groups of hepatic cords, especially in the centre of the lobule, were separated from each other by intralobular œdema, giving rise to a false impression of cirrhosis, but there is no typical pseudo-lobulation.

Case III.—The liver weighed 46 oz.; surface dark brownish in colour; normal in size; capsule thickened; firm in consistency and cuts with resistance; section dark brown in colour; lobular markings indistinct.

Microscopically—no loss of lobular pattern; no increase of fibrous tissue in any of the ramification of the Glisson's capsule; the sinusoids were plugged with dark brown pigment, which is especially seen in the Kupffer cells.

Case IV (Plate VIII).—The liver weighed 48 oz.; slightly enlarged in size; the capsule thin; the surface greyish in colour; cut section moist and slaty in colour; lobular markings indistinct.

Microscopically—no fibrosis in the organ; Kupffer cells loaded with malarial pigment; no loss of lobular pattern.

Case V.—Chronic malarial liver (Stanley Medical School specimen).

Microscopically—sinusoids congested; Kupffer cells loaded with dark malarial pigment; sporadic fatty change; no loss of lobular pattern; fibrosis not marked; slight widening of the portal spaces; focal necroses of the hepatic cords.

Case VI.—Chronic malarial liver (Stanley Medical School specimen).

Microscopically—no loss of lobular pattern; slight widening of the portal spaces; hepatic cords contracted but in places show cloudy swelling; Kupffer cells contain dark brown malarial pigment in moderate amount; slight pericholangitic fibrosis.

PLATE VII.

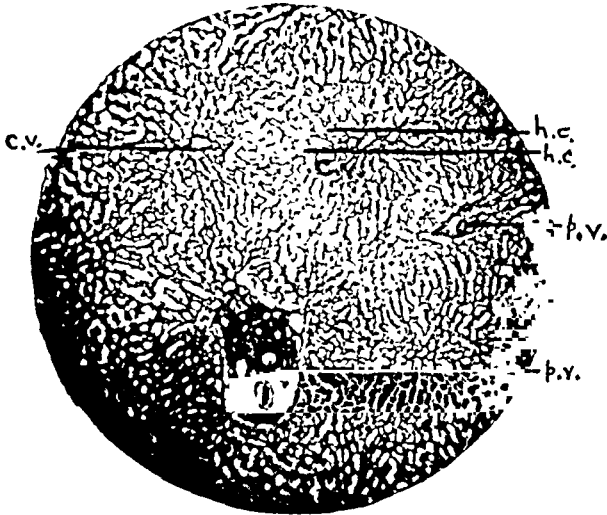


FIG. 1. *Case I.*—Photomicrograph of the reticulum of the liver showing the normal pattern of the hepatic parenchyma and the absence of fibrosis. The hepatic cords (*h.c.*) are unstained and are represented by the empty spaces in the figure.

p.v.—portal space; *c.v.*—central vein. (Foot and Ménard's silver carbonate impregnation technique.)



FIG. 2. *Case I.*—Photomicrograph of the section of the liver showing the sinusoids which contain Kupffer cells (*a*) loaded with malarial pigment.

h.c.—hepatic cords. (Ehrlich's acid hæmatoxylin and eosin.)

PLATE VIII.

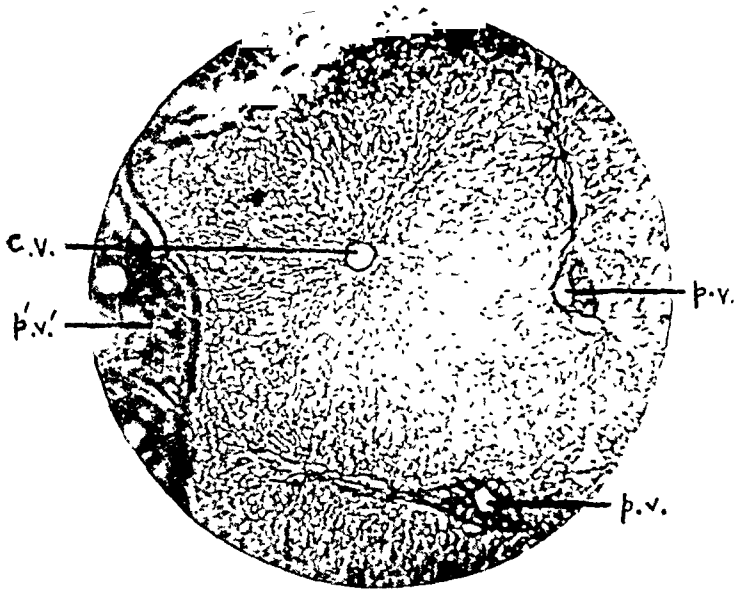


FIG. 3. *Case IV.*—Photomicrograph of the reticulum of the liver showing the normal architecture of the hepatic parenchyma and the absence of fibrosis. The hepatic cords are unstained and are represented by the empty spaces in the figure.

p.v.—small portal space; *p'.v'*.—portal space of the third order; *c.v.*—central vein. (Foot and Mênard's silver carbonate impregnation technique.)

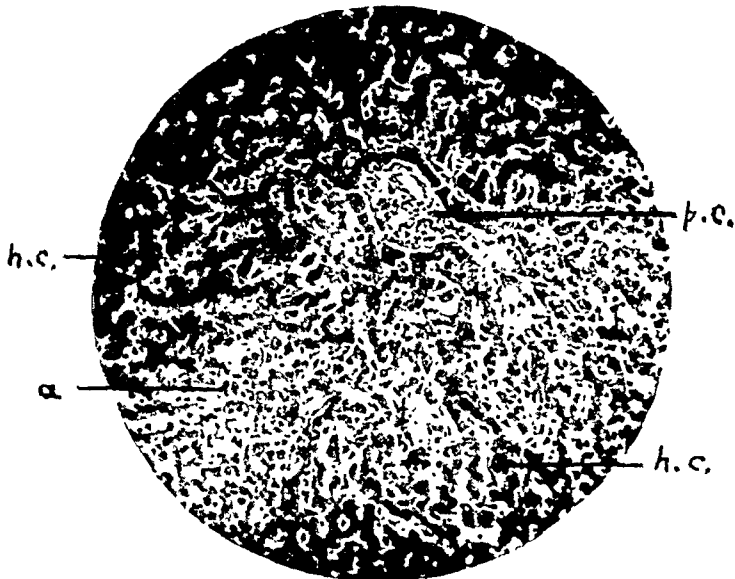


FIG. 4. *Case IV.*—Photomicrograph of the section of the liver showing the Kupffer cells (*a*) loaded with dark brown malarial pigment.

p.c.—portal space; *h.c.*—hepatic cords. (Ehrlich's acid hæmatoxylin and eosin.)

It may be noted from the above description that the *important histopathological features* in the liver in this series are : absence of any loss of lobular pattern ; loading of Kupffer cells with malarial pigment ; and no noticeable increase of fibrous tissue in the liver. Sporadic fatty degeneration and focal necroses of the hepatic cords, slight sclerotic widening of the portal spaces and congestion of the sinusoids were some of the other, but inconstant, findings. Endo-phlebitis of the collecting veins of the hepatic venous tree and pericholangitic fibrosis were occasional and rare findings. *In none of the cases investigated was there any pseudo-lobulation or fibrosis similar to that seen in a typical case of cirrhosis of the liver.*

DISCUSSION.

The indiscriminate use of the term 'cirrhosis' to any and every type of fibrosis of the liver, has no doubt added to the confusion regarding the rôle of malaria in cirrhosis of the liver. The term 'cirrhosis' was first invented by Laennec in 1819 to denote the condition of portal cirrhosis (now named after him) in which the following features stand out prominently : (1) necrosis of the hepatic parenchyma ; (2) replacement (residual) fibrosis ; (3) atypical regeneration of the hepatic parenchyma (pseudo-lobulation) and (4) involvement of the whole organ. Elsewhere (Ramachandra Rao, 1933 ; Radhakrishna Rao, 1935*a* and *b*) ; Ramachandra Rao and Radhakrishna Rao, 1934) it is pointed out that the word 'cirrhosis' should be limited to an organ showing all the above-mentioned features. Though it is out of place here to discuss the definition and classification of cirrhosis of the liver, we wish to point out that the word 'cirrhosis' should only be used to the portal and toxic types of hepatic fibrosis, as these alone exhibit all the features mentioned above.

Judging from the above standards, none of the cases studied in this series showed a true cirrhotic condition of the liver. None of the specimens exhibited any loss of lobular pattern. Except slight widening of the portal spaces, the fibrosis in the liver was negligible. Though in all the instances the Kupffer cells were loaded with malarial pigment, the silver impregnation of the reticulum of the liver showed absence of marked fibrosis. In some of the hæmatoxylin and eosin stained sections, a division of the parenchyma into small, irregular islands by intralobular œdema simulated pseudo-lobulation ; but the silver impregnation of the reticulum of the same specimen showed only a normal hepatic architecture.

As pointed out by Rolleston and McNec (1929), the possibility of slight necrosis of the hepatic parenchyma in acute or sub-acute malaria cannot be denied, but the liver possesses a remarkable capacity to regenerate (Mann and Bollman, 1926) so that such necrotic changes leave no permanent trace behind. In almost all the instances in this series, the chronic malarial liver showed no increase of fibrous tissue.

The causes of cirrhosis of the liver are so many that each case should be thoroughly studied in order to arrive at the ætiological agent responsible for the disease. One of us (Radhakrishna Rao, 1933) showed from an intensive investigation of cases of cirrhosis of the liver in the King George Hospital, Vizagapatam, that malaria 'cannot be considered to be a causal factor in the production of cirrhosis of the liver, though it may be an important predisposing cause'. It is

important to remember that malaria and cirrhosis of the liver may coexist in the same patient without the former exerting any influence on the latter condition.

Finally, it may be said that the evidence presented in this paper proves that malaria *per se* is not a direct cause of cirrhosis of the liver.

SUMMARY.

In a previous communication (Tirumurti and Radhakrishna Rao, *loc. cit.*) it was pointed out that the common association of malaria and cirrhosis of the liver in India has given rise to divergence of opinion regarding the rôle of the former in the causation of the latter. A critical investigation of the morbid histological changes in chronic malarial livers by the application of different staining methods including the silver impregnation of the reticulum has confirmed our previous view that malaria *per se* is not a direct cause of cirrhosis of the liver.

ACKNOWLEDGMENTS.

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CARCINOMA OF THE THYMUS.

BY

AMAR NATH GOYLE, M.B., PH.D.,

A. VASUDEVAN, M.B., B.S.,

AND

K. G. KRISHNASWAMY, M.B., B.S.

(From the Department of Pathology, Medical College, Madras.)

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SEVERAL cases of tumour of the thymus have been reported within recent years. Much confusion still exists as to their origin and true nature and this is chiefly due to the fact that there is no general agreement concerning the origin and nature of the thymus. Recently, we had to report on a thymus tumour which was removed at the General Hospital, Madras, and this case forms the basis for this paper and affords an opportunity for discussing the malignant tumours of this gland. The subject of the growth was a Hindu boy of thirteen years of age and the tumour formed a mass situated in the lower part of the neck extending on either side to the posterior margin of the sternomastoid muscle. This was removed at operation, which was unfortunately fatal, and has been examined in detail.

HISTOLOGICAL APPEARANCES (Plate IX, figs. 1 to 4).

Under the low power of the microscope there could be made out at the periphery of some of the sections a capsule of loose connective tissue fibres containing a few cells. The tumour appeared as a solid mass of cells, closely packed together. At one part, the section showed thyroid tissue in contact with the tumour cells (Fig. 1). A definite fine reticulum was seen in sections stained with Foot and Ménard's (1927) silver carbonate impregnation method, and the reticular fibres were intimately associated with the cells (Fig. 2). In places where the reticulum was particularly dense it appeared to divide the growth into small irregular areas. Under high power the cells showed a tendency to alveolar arrangement. According to their histological characters the cells could be divided into several types. The type of cells which predominated were small in size measuring 5μ with nuclei of 3μ diameter

EXPLANATION OF PLATE IX.

- Fig. 1. Photomicrograph of a section of the growth with low magnification, showing a diffuse mass of closely packed cells. At one place thyroid tissue is seen in contact with the proliferating cells.
- „ 2. Photomicrograph of a section of the growth stained with Foot and Mènard's silver carbonate impregnation method to show the reticular fibres. Note their intimate relationship with the neoplastic cells.
- „ 3. Photomicrograph of a section of the tumour under high power showing the characters of cells. Note the small size of the cells with dense nuclei and reticulated cytoplasm. The polyhedral outline of some of the cells can be made out. Four larger somewhat oval cells with vesicular nuclei are also seen in this section. A mononucleated giant cell is seen in the centre of the field.
- „ 4. Shows an abortive Hassall's corpuscle.



Fig. 1.

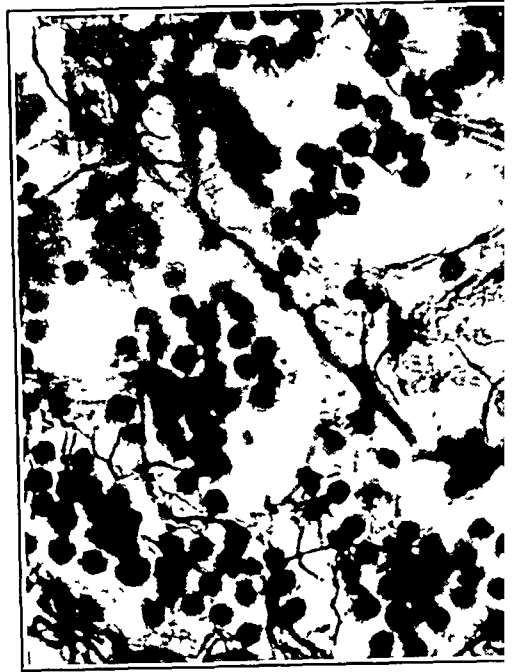


Fig. 2.

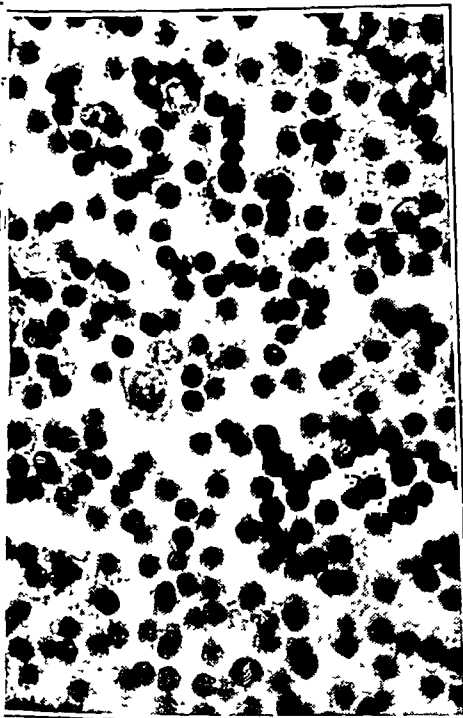


Fig. 3.

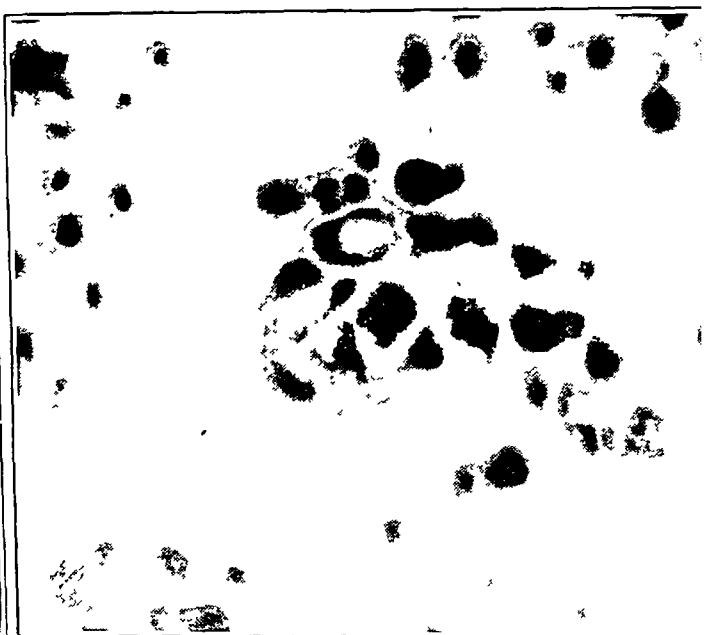


Fig. 4.

(Fig. 3). The cytoplasm was as a rule moderate in amount and acidophilic in reaction and generally deeply stained, but in some of the cells the cytoplasm was abundant and the nucleus was situated eccentrically, and in others it was only present as a narrow rim round the nucleus. The cells, as a rule, were slightly separated from one another and not mutually compressed. Their outline was polyhedral and the cytoplasm appeared to throw off protoplasmic processes which occasionally continued into fibrils. The epithelial structure of the cells was clearly seen under the one-twelfth oil immersion lens. The nucleus was usually compact, round and hyperchromatic. Mitotic figures were not observed. The mass of the tumour was made of these cells, but scattered among them were a few larger cells the cytoplasm of which was slightly acidophilic and small in amount compared to the size of the nucleus. These cells varied in size, were not polyhedral in shape but oval and irregular. The nuclei of these were oval in shape and usually vesicular and larger and less deeply stained than those of the smaller cells mentioned above (Fig. 3). The chromatin was irregularly distributed and collected in clumps. These cells would appear to correspond to the reticular cells of mesenchymal origin.

Occasionally one could see a giant cell with or without masses of chromatin, representing remnants of ingested cells.

Scattered all through the sections were small, spheroidal groups of concentrically arranged cells with large, somewhat hyperchromatic nuclei. These cells occurred in groups of three or four, or in larger aggregations. They were rather denser than the other elements of the tumour, tended to show the appearance of early keratinization, particularly about their periphery, and all the characteristics of young thymic corpuscles in an early stage of development. Many of them appeared to lie in free spaces in the alveoli of smaller tumour cells (Fig. 4).

The tumour cells showed no tendency to undergo degenerative changes. Necrosis was not observed. There were no plasma cells nor eosinophile cells.

The stroma was made up of thin strands of connective tissue and there were well developed blood vessels.

Histological diagnosis.—That the tumour has originated from the thymus is shown by the presence of Hassall's corpuscles though these are of a rudimentary nature. The polyhedral outline of the neoplastic cells and their tendency to form alveoli point to the epithelial nature of the tumour. The presence of abundant argentophil reticular fibres which are intimately associated with the neoplastic cells is indicative of their reticular nature. It is thus clear that this case belongs to the group of carcinomas derived from the epithelial reticular cells of the thymus. A strange fact noted in this tumour is the presence of dense deeply staining nuclei in which no structure could be made out. In this respect the present case differs from those already reported in which the nuclei of the tumour cells have been described as more or less vesicular with chromatin network and one or more nucleoli. The small size of cells is another noteworthy feature. The absence of karyokinetic figures and of any variation in the shape and size of the cells suggests that the tumour was of a relatively low grade of malignancy though it is not possible to say if there were any secondary deposits in the other organs as no post-mortem examination was made.

COMMENT.

Origin and nature of the cells of the thymus.

As mentioned above, it is extremely difficult to classify tumours of the thymus. In order fully to comprehend the nature of these tumours, it is necessary to consider some points in the histology of this organ. The thymus is originally an epithelial structure, being an out-growth of the third branchial cleft on each side of the medial line, and consisting of several layers of cylindrical epithelium. At an early stage of development, this epithelial cell mass is invaded by lymphocytes.

The cortical thymus consists of densely packed masses of small round cells. These are called thymocytes and are morphologically identical with lymphocytes of the lymphoid tissue. Scattered between these cells are found elongated reticular cells with pale round or oval nuclei. The medulla consists of reticular cells similar to those of the cortex. It also contains Hassall's corpuscles—the characteristic structures of the thymus. The lymphocytes are much less numerous here than in the cortex.

It seems to be agreed that the Hassall's bodies and the vast majority of reticular cells are of entodermal origin. Hassall's corpuscles are formed from hypertrophic or degenerating reticular cells. A few reticular cells of mesenchymal origin are found in the vicinity of blood and in the capsule. The reticular epithelial cells become flattened from infiltration with lymphocytes and cannot be easily distinguished from the nuclei of the connective tissue reticular cells but the former do not take up any of the dye-stuff in vitally stained animals in contrast to the reticular cells of mesoblastic origin.

There is, however, considerable dispute as to the origin and nature of the small round cells of the thymus. Are these lymphocytes differentiated entodermal cells or are they derived from mesodermal tissue? Some histologists, Schridde (quoted by Kaufmann, 1929), for example, consider that the thymus is purely an epithelial organ and that the thymocytes or lymphocytes are of entodermal origin and that a true thymus tumour has an epithelial structure. According to this view a new growth of this region composed of lymphoid cells is a lymphosarcoma which has its origin in the lymphatic glands of the anterior mediastinum and is not of thymic origin at all. This view appears to have lost much ground since Maximow (1931) showed that the small round cells of the thymus were of mesodermal origin. Not only are they morphologically identical with the lymphocytes but they are equally susceptible to X-ray injury and are identical in their serological reactions. They both show the same type of amoeboid movement. Furthermore, the thymocytes are liable to be transformed into plasma cells and eosinophile myelocytes as are the lymphocytes. According to him these cells migrate into the thymus from outside and invade the primitive epithelial cell mass and multiply there. In this way, the original epithelial sheet is converted into a reticular cell mass. Many of these lymphocytes, no doubt, arise from the perivascular mesenchymal cells of the blood vessels of the thymus. This view may be said to hold the ground at present and we have reasons to oppose it. This preliminary statement of the origin and nature of the various cell types composing the thymus, we hope, makes the ground clear for a discussion of the various points of origin of the malignant tumours of the thymus.

Histogenesis of the thymus tumours.

Tumours of the thymus may arise from the following types of cells:—

- (A) Reticular cells of entodermal origin of the medulla and cortex.
- (B) Hassall's corpuscles which are also of entodermal origin.
- (C) Lymphocytes of mesenchymal origin.
- (D) Reticular cells of mesenchymal origin.
- (E) Interlobular connective tissue and the capsule.

We shall now consider the tumours arising from these structures in greater detail.

A and B. Reticular cells of entodermal origin and Hassall's corpuscles.—Both these types of cell structures are epithelial in origin and the tumours derived from them would be called carcinomata. Several of these have been described: new growths resembling epitheliomas containing more or less developed Hassall's bodies or epithelial pearls, and tumours composed of epithelial cells suggesting an origin from the entodermal reticular cells of the thymus with or without rudimentary Hassall's bodies, and typical adenocarcinomas. Recently, Schuster (1927) has described a mucus-secreting glandular carcinoma of the thymus. The vast majority of these epithelial tumours appear to have originated from the reticular cells. There is no authentic record of a carcinoma of the thymus arising from Hassall's bodies and consisting of these structures alone. Multinucleated giant cells are usually seen in these tumours and they are probably formed by the rapid division of the nuclei of the reticular cells without a corresponding division of the cytoplasm.

C. Lymphocytes or thymocytes.—The tumours which originate from these elements are practically indistinguishable from the lymphosarcomata of the lymphoid tissues. These are composed of uniformly small round cells and are devoid of reticular fibres and Hassall's corpuscles. It appears, however, doubtful if a case of pure lymphocytoma of the thymus has ever been described, but leukæmic infiltrations do occur which may be mistaken for neoplasms of this type. In some of these cases reversion of the lymphocytes to the primitive type cell may be so pronounced that the histological features resemble very closely those of a large round cell sarcoma. Simmonds (quoted by Kaufmann, *loc. cit.*) suggests the name 'thymoma' for tumours arising from these cells. This view is of course based on the assumption that the thymocytes are peculiar to this gland and are of epithelial origin. But as these cells are now regarded by many workers as derivatives of mesoblastic elements which wander into the primitive epithelium of the thymus during its early development, this designation is unsatisfactory. A much better term is 'lymphocytoma' and it is better to avoid the term 'thymoma'. On the contrary, Jaffé (1926) is of the opinion that the term 'thymoma' should be restricted to tumours arising from the epithelial elements of the thymus gland containing Hassall's bodies. We share this view though we do not attach much importance to the presence of Hassall's bodies. These structures, it appears from a study of the literature, are not always present. If the term 'thymoma' is to be used at all, it should denote a tumour of the thymus of undoubted epithelial origin, i.e., arising from those cells which constitute the essential elements of this organ. It cannot be used to designate a tumour which has its origin in lymphocytes of mesoblastic origin which really do not belong to the organ but only migrate into it from outside and are neither characteristic nor peculiar to the thymus tissue.

D. Reticular cells of mesenchymal origin.—A tumour arising from these cells may be termed lymphosarcoma or large round cell sarcoma. The most suitable name, however, is reticular cell sarcoma. Most of the cases reported in the journals as lymphosarcoma of the thymus appear to have been derived from these cells. It may be noted that tumours arising from the reticular cells of entodermal origin show epithelial characters, while tumours of reticular cells of mesenchymal origin exhibit mesoblastic features, namely, irregularity of cellular outline, tendency to the formation of spindle cells, etc.

E. Interlobular connective tissue and the capsule.—A tumour arising from these tissues has the characters of a spindle-cell sarcoma. Two such cases have been reported by Simmonds (quoted by Kaufmann, *loc. cit.*). According to Ewing (1928) the origin of these tumours has never been completely traced to the connective tissue stroma and he is inclined to the view that the so-called spindle-cell sarcomas are really varieties of thymoma, i.e., tumours derived from the lymphocytes or the reticular mesenchymal cells of the thymus. Foot (1926) thinks that no such tumour has ever been described and he sounds a note of warning against regarding tumours composed of cells resembling large spindle cells as fibrosarcomas inasmuch as these cells may have been derived from entodermal reticular cells and are therefore of epithelial origin. In doubtful cases, the presence of Hassall's corpuscles even though of a rudimentary nature may indicate an entodermal origin.

Relative incidence of malignant tumours of the thymus.

In studying the reports of tumours of the thymus since 1926, we were greatly impressed with the fact that almost all the reported cases were of the carcinomatous type. We mention this because statements are frequently made in textbooks and journals that sarcomata are the commonest type of tumours found in the thymus. Rubaschow (quoted by Foot, *loc. cit.*) compiled a list of thymus tumours in 1911 and found 52 sarcomas, 12 carcinomas and 11 miscellaneous tumours. Excluding the miscellaneous tumours there were reports of 81 cases of thymus tumours up to 1926. According to Symmers and Vance (1921) only four cases of primary carcinomata had been reported up to the time they published their paper. Bringing the figures up to date, Foot stated in 1926 that his case was the seventh. Brannan (1926) reviewed the literature up to 1926 and found 15 authentic cases of carcinoma of the thymus. It will be noted that there is a considerable difference between the figures of Foot and Brannan but the fact remains that these authors and many others hold that the thymus carcinomata are of rare occurrence. It also emphasizes the extreme difficulty of determining the origin and nature of thymus tumours. Ewing says that lymphosarcoma is the most frequent form of thymus tumour. We have read the cases which have been reported or abstracted in the *Archives of Pathology* and other available journals since 1926 and have been able to collect 25 cases of tumours arising from the thymus region, which are as follows: Foot (1926), Brannan (1926), Brown (1926), Schuster (1927), Kaijser (1928—two cases), Meeker (1928), Matras and Priesel (1929—seven cases), Parabutschew (1930—four cases), Duguid and Kennedy (1930—two cases), Leyton, Turnbull and Bratton (1931—two cases), Craver (1931), Babès (1932), and McDonald (1932). We do not claim that the list is complete, but in our opinion it gives a fair idea of the incidence of malignant tumours of the thymus. From a study of these, it appears that there

were only two cases diagnosed as lymphosarcoma of the thymus during the period under review, but both of these had their origin in the mediastinal lymph nodes (Brown's case and Duguid and Kennedy's second case). There were, however, a few cases of mixed type in which both epithelial and lymphoid elements were present. We refer to the cases of Matras and Priesel and of Babès (quoted by McDonald, 1932), who classify thymus tumours on the presence or otherwise of lymphocytes and their arrangement. Such attempts have added to the confusion of an already difficult and complex subject. It appears doubtful whether the presence of lymphocytes in an epithelial tumour of an organ such as the thymus which is normally rich in these cells is of much significance. Moreover, as the growth of a reticular cell carcinoma proceeds the lymphocytes naturally tend to decrease in number and ultimately may disappear altogether, so that at different times and in different sections the proportion of the lymphocytes to the tumour cells (epithelial reticular cells) may vary considerably. Meeker's case also presents some difficulty as to its origin but according to the author the tumour cells showed a more or less close resemblance to the small thymus cells and the thymus reticular elements. Excluding cases of doubtful origin and nature there were 19 cases of primary carcinoma of the thymus. We have not been able to find a single case of undoubted primary lymphosarcoma of the thymus during the period under review. It is thus evident that since 1926 the tendency to diagnose carcinoma of the thymus has been very marked.

Is it not possible that some of the tumours diagnosed and reported as lymphosarcomas in the earlier literature represented an extension of the neoplastic process from lymph nodes of the anterior mediastinum to the thymus? A case of this type was recently described by Brown. It was a reticular cell lymphosarcoma of the thymus region, the origin of which was traced to the lymph nodes of the anterior mediastinum.

The possibility of a transformation of the Hodgkin's granuloma into a sarcomatous process has also to be kept in mind. That sarcomatous change may occur in the terminal stages of Hodgkin's disease is well known and cases of this type are not uncommon. Such a condition is called Hodgkin's sarcoma. It is conceivable that some of the cases described as lymphosarcoma of the thymus or thymoma in the literature are of this nature.

It may also be noted that Hodgkin's granuloma has been known to take on invasive characters. Shennan (1928) has described four tumours of the thymus composed of cells like those of lymphadenoma and he calls such tumours 'thymoma of lymphadenoma type'. They may either invade the surrounding tissues or may remain localized. There are, however, no convincing reasons for regarding these cases as lymphosarcomas of the thymus. In view of all these considerations we are inclined to believe that carcinomata of the thymus are not so rare compared with thymus sarcomata as is generally believed.

SUMMARY.

A case of carcinoma of the thymus arising from the epithelial reticular cells is described. It is composed of epithelial cells of small size showing a tendency to alveolar arrangement. Though well-developed Hassall's corpuscles were not found

aggregations of cells bearing resemblance to these structures in their early development were noticed.

A brief discussion on the origin and nature of malignant tumours of thymus is added. The suggestion is made to restrict the term 'thymoma' to tumours of the thymus which arise from the epithelial reticular cells.

The question of the relative incidence of thymus carcinoma and sarcoma is discussed with the conclusion that thymus carcinoma is not so rare compared with thymus lymphosarcoma as is generally believed.

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A DIET SURVEY OF SOME FAMILIES AND INSTITUTIONS IN CALCUTTA.

BY

H. ELLIS C. WILSON, M.B., CH.B., D.SC.,

Professor of Biochemistry and Nutrition,

BASHIR AHMAD, PH.D.,

Assistant Professor of Biochemistry and Nutrition,

AND

DWIJENDRA NATH MULLICK, M.SC.

*(From the Department of Biochemistry and Nutrition, All-India Institute
of Hygiene and Public Health, Calcutta.)*

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A COMPLETE diet survey in India is probably the most urgent and to-day should be the most fruitful field of investigation in the different lines of nutritional research. In the first place in few large areas of the world are there such a diversity in food habits due to social, economic and climatic conditions. Further in many cases certain types of disease are found in association either with certain areas or social groups. It is probably no exaggeration to say that there is a large and unexplored mass of material demanding a survey both dietetic and clinical which would be of value in assessing the precise rôle of nutrition in relation to disease. This expectation is enhanced by the impression the senior author (H. E. C. W.) has obtained from medical men in practice in India that malnutrition is, in their opinion, much more widespread and probably more complex than was hitherto realized. In the second place any improvement in the diet of the masses as a result of the survey must be a slow process and indeed rather in the nature of an expedient, hence if it is to be carried out without waste must depend on accurate quantitative and qualitative knowledge of the actual diet consumed and that actually or potentially available.

The present investigation was undertaken primarily as an initial step to correlate diet with physique and the incidence of clinical signs of disease. The field studies do not unfortunately include the poorer classes, nevertheless it can be taken that the diets recorded here are better and probably much better than what is consumed by the masses. It is hoped to obtain information on this class but for various reasons accurate data are difficult to procure. The present study includes ten middle-class Bengali Hindu families, a Bengali male hostel, two orphanages—one Muslim and one Hindu, an Anglo-Indian school, a Marwari gate-keeper and an Oriya sweeper

(the latter may be considered as representative of the poorer classes). The data on the families were collected by one of us (D. N. M.) who knew them personally and obtained figures concerning the food purchased and the age, number and sex of the members of each household. As regards the institutions, we had in each case the active co-operation of the managing staff who could supply us with the exact details of the food purchased and dispensed to the inmates each day. The latter data could be relied on as in every case the budget was limited and every attempt was made to make the most of the money available. The cost of the diet per head and per man value (henceforth M. V.*) has been calculated at the current prices in Calcutta which it is hoped should serve as a guide as to what is possible for different classes of the community according to the income received. The income of the head of the household has also been given. This, unfortunately except as a rough index of their economic status, is not of much value in individual cases as the junior members of the household were often contributing their share in the food budget. The Bengali male hostel was probably a fairly good guide to the upper standard of living of unmarried wage earners of Rs. 40 per month with other obligations.

In this communication the composition of the diet has been analysed under the following heads: protein, animal protein, fat, animal fat, carbohydrate, ash, calcium, phosphorus, total calories, per cent of calories from dairy products and cereals respectively, and the per cent of the food budget spent on fruits and vegetable and dairy products. An analysis and discussion of the diets as regards vitamins will be made when the figures for the most important ones have been completed. In the Table are collected the data for each family, institution and individual. As the families are all Hindu and of the same social class they have been averaged and this figure compared with the other groups for comparison. The figures employed in the analysis of the diets were taken, as regards the major food-stuffs, for the most part from McCarrison's (1929) 'Food', while those for the minerals were derived from analysis carried out in this laboratory.

Calories.—The average calorie intake per M. V. ranges with two exceptions (one family and the gate-keeper) from 2,400 to 3,800 per day. In the case of the family with over 5,000 calories there was probably a certain amount of waste or leakage and in addition their food budget and income were high. The Marwari gate-keeper was an athlete (wrestler, stood 6' 3") and is hence not quite a typical sample. The data concerning the families are probably less accurate than the others owing to less careful management of the household. The data concerning the sweeper are probably most open to criticism but it is the general impression of the two senior authors (H. E. C. W. and B. A.) that it could scarcely be much better at least as regards quality. The calorie intake is in all cases up to that (2,400) recommended by the League of Nations (1935) as the minimum for a sedentary worker. In most cases, however, they fall short of that (3,400) recommended by the Committee of the British Medical Association (1933) and that (3,000) by the Joint Committee of the Ministry of Health (1934). It is expressly stated in the League of Nations' report that their figures refer to those living in a temperate climate and it would seem likely in view of the frequent necessity in India of planning diets for famine relief and possible future economic legislation that some standard should be sought. The figures for the orphanages are of interest in this respect.

* Lusk's co-efficients.

TABLE.

No.	Name.	MAJOR FOOD-STUFFS.					MINERALS.			CALORIC DISTRIBUTIONS.					MONETARY DISTRIBUTIONS.		
		Total protein, g.	Total fat, g.	Total carbohydrate, g.	Total animal protein, g.	Total animal fat, g.	Total ash, g.	Total phosphorus, g.	Total calcium, g.	On protein, per cent.	On fat, per cent.	On carbohydrates, per cent.	On cereals, per cent.	On dairy products, per cent.	Total cost in annas.	On vegetable and fruits, per cent.	On dairy products, per cent.
1	A. FAMILIES.																
	Bengali Hindu family. Approx. income Rs. 500. Heads 5.125 : M. V. 4.033.	4,663	187.46	592.46	40.51	90.61	21.90	2.98	1.54						11.06		
	Per day per head ..	5,924	238.17	752.74	62.90	115.13	27.82	3.78	1.96						14.05		
2	Bengali Hindu family. Approx. income Rs. 500. Heads 14.0 : M. V. 11.85.	2,156	51.98	283.77	23.42	45.67	7.32	0.98	0.93						5.52		
	Per day per head ..	2,547	61.27	335.26	27.07	53.96	8.65	1.15	1.11						6.52		
	Per day per M. V. ...																
3	Bengali Hindu family. Approx. income Rs. 500. Heads 15.0 : M. V. 12.69.	1,818	47.22	256.27	10.53	38.46	6.741	1.021	0.589						4.62		
	Per day per head ..	2,149	55.84	302.91	23.09	45.46	7.96	1.207	0.696						5.46		
	Per day per M. V. ...																

*M. V. = Mean value.

TABLE--contd.

No.	Name	Total calories.	MAJOR FOOD-STUFFS.					MINERALS.			CALORIC DISTRIBUTIONS.						MONETARY DISTRIBUTIONS.		
			Total protein, g.	Total fat, g.	Total carbo- hydrate, g.	Total animal protein, g.	Total animal fat, g.	Total ash, g.	Total phosphorus, g.	Total calcium, g.	On protein, per cent.	On fat, per cent.	On carbohydrates, per cent.	On cereals, per cent.	On dairy products, per cent.	Total cost in annas.	On vegetable and fruits, per cent.	On dairy products, per cent.	
4	A. FAMILIES— <i>contd.</i>																		
	Bengali Hindu family. Approx. income Rs. 500 p.m. Heads 8: M. V. * 7.49.	3,943	107.97	117.16	588.2	47.91	63.79	16.2	2.20	1.02	11.22	27.63	61.14	49.37	13.75	12.9	41.79	21.48	
	Per day per head .. Per day per M. V.	4,212	115.3	125.14	628.2	51.17	63.13	17.3	2.35	1.09						13.8			
5	Bengali Hindu family. Approx. income Rs. 300. Heads 12.0: M. V. 9.59.																		
	Per day per head ..	2,790	58.43	68.85	466.11	17.33	13.21	9.64	1.06	0.37	8.58	22.91	68.47	60.86	2.44	3.04	19.94	15.41	
	Per day per M. V.	3,492	73.12	86.15	583.25	21.69	16.53	12.06	1.32	0.47						3.806			
6	Bengali Hindu family. Approx. income Rs. 300. Heads 12.0: M. V. 10.16.																		
	Per day per head ..	1,673	33.62	39.94	283.87	7.36	13.95	5.186	0.747	0.397	8.24	22.15	60.00	65.80	10.65	2.447	27.38	38.02	
	Per day per M. V.	1,974	30.71	47.03	335.28	8.69	16.48	6.125	0.882	0.169						2.800			

7	Bengali Hindu family. Approx. income Rs. 200. Heads 6.0 : M. V. 4.9.	3,145	73.97	63.44	549.3	6.69	17.23	12.18	1.257	0.423	9.04	18.7	71.6	62.20	5.58	5.40	44.7	12.33
	Per day per head	3,850	90.57	77.60	672.6	8.19	21.13	14.92	1.539	0.519						0.61		
	Per day per M. V.																	
8	Bengali Hindu family. Approx. income Rs. 60. Heads 7.0 : M. V. 6.02.	2,793	54.07	83.81	437.04	12.76	10.88	7.905	1.025	0.313	7.93	27.90	64.15	50.00	7.81	4.19	29.30	15.60
	Per day per head ..	3,248	62.87	97.45	508.19	14.83	23.11	9.192	1.192	0.364						4.87		
	Per day per M. V.																	
9	Bengali Hindu family. Approx. income Rs. 60. Heads 11.0 : M. V. 9.52.	2,913	64.30	75.66	474.50	20.66	21.69	8.953	1.159	0.383	9.05	24.19	66.79	56.27	6.87	4.26	40.26	23.2
	Per day per head ..	3,366	74.29	87.42	548.35	23.87	25.07	10.348	1.339	0.443						4.92		
	Per day per M. V.																	
10	Bengali Hindu family. Heads 9.0 : M. V. 7.56.	2,912	67.11	93.50	474.70	23.27	20.6	7.96	1.225	0.679	9.78	21.00	69.21	66.57	7.44	3.13	16.83	31.01
	Per day per head ..	3,348	79.89	75.70	565.14	28.21	24.55	9.48	1.458	0.809						3.73		
	Per day per M. V.																	
	Average for families.																	
	Per day per head ..	2,871	67.83	84.54	440.62	22.84	34.51	10.40	1.365	0.664	9.55	26.96	63.48	54.48	12.99	5.66	29.21	28.43
	Per day per M. V. ..	3,411	80.59	100.67	523.19	27.03	40.96	12.39	1.622	0.793						6.67		

*M. V. = Man value.

No.	Name.	Total calories.	MAJOR FOOD-STUFFS.				MINERALS.			CALORIC DISTRIBUTIONS.					MONETARY DISTRIBUTIONS.			
			Total protein, g.	Total fat, g.	Total carbohydrate, g.	Total animal protein, g.	Total animal fat, g.	Total ash, g.	Total phosphorus, g.	Total calcium, g.	On protein, per cent.	On fat, per cent.	On carbohydrates, per cent.	On cereals, per cent.	On dairy products, per cent.	Total cost in annas.	On vegetable and fruits, per cent.	On dairy products, per cent.
11	B. INSTITUTIONS.																	
	Muslim Orphanage. Heads 202-16; M. V.* 164-00.																	
	Per day per head ..	2,203	72-96	31-75	392-37	8-62	3-12	10-16	1-40	0-40					2-18			
	Per day per M. V. ..	2,702	89-49	38-94	481-25	10-58	3-83	12-47	1-72	0-49					2-68	17-1	0	
12	Hindu Orphanage. Heads 20-8; M. V. 20-8.																	
	Per day per head																	
	Per day per M. V.	2,783	65-10	42-64	517-07	10-72	10-85	7-96	1-25	0-25					2-96	0-53	0-05	
13	Bengali Male Hostel. Income Rs. 40 per head, Heads 16-0; M. V. 16-0.																	
	Per day per head																	
	Per day per M. V.	2,499	51-17	51-97	440-4	13-65	12-47	6-55	0-001	0-25					3-51	0	0	

As mentioned above the data were presented us from entirely independent sources and without any bias on our part except that of emphasizing the importance of accuracy. The boys appeared active and free from disease and in all cases their appetites were satisfied. They were also weighed regularly by the authorities. Detailed data as regards height, weight, physique by the A. C. H. (arm, chest, hip) index of nutrition are being collected and will be published later. It will be noted that the calorie intake per M. V. all fell remarkably close at 2,800 which is all the more interesting in that, as regards the Hindu orphanage, the M. V. and per head value were identical, the boys being all over 14 years of age. The proposal to accept the calorie intake of active boys who are subjectively satisfied as regards quantity would be a not unreasonable figure to use as a safe or even maximum figure for men doing light work in this country.

Protein.—The protein element in nutrition in India may ultimately prove to be the most important and it certainly will be the most difficult to remedy. At the outset of this discussion a quotation of a few of the more recent Western standards should not be out of place. The British Medical Association Committee recommended 100 g. of protein of which 50 g. should be of good quality (animal sources). This would constitute about 12 per cent of the total calories of their diet. The Ministry of Health Committee modified the animal protein to 37 g. and accepted a total protein intake of 100 g. This would constitute 13 per cent of the total calories. As regards children the protein requirements are somewhat greater relatively than adults and should hence constitute a greater percentage of the total calorie intake.

As regards the protein intake of the families this is found to vary from 39 g. to 115 g. per day per M. V. with an average of 80 g. of which 27 g. are from animal sources. As the average calorie intake is relatively high the protein calories constitute only about 9 per cent of the total, a figure which falls below those recommended above, namely 12 per cent to 15 per cent. It should be pointed out, however, that any waste is much more likely to affect the cheaper foods such as rice which is poor in protein. This would tend to increase the percentage of calories derived from this source without decreasing the total to any considerable extent. The institutions are somewhat above or below what is consumed by the families as regards the protein intake, while the animal protein is exceptionally low, 9 g. to 13 g. per M. V. in the two Indian institutions and 29 g. per M. V. in the Anglo-Indian school. The percentage of calories derived from protein is not above and in the case of the Hindu orphanage rather below the standard. As these are children's institutions it will be appreciated that the protein intake is probably definitely below optimum in view of the fact that the relative requirements of young people for this food-stuff are greater than for adults. As regards the Marwari gate-keeper who is hardly a true representative sample his total protein intake is 144 g. of which, however, only 5.2 g. come from animal sources. His staple cereal is wheat with dhal and only occasionally was rice taken. The Oriya sweeper is probably fairly typical of his class. He is living on 44 g. of protein a day which is about the lower level recommended by Chittenden. This protein is for practical purposes almost entirely derived from cereals, such as rice, which in addition is relatively poorly absorbed. It is difficult to see how real health could be maintained over a long period on such a diet. The male adult hostel presents a poor picture and is probably fairly representative of the diet of a class earning not more than Rs. 40 per month. It falls

short of the Western standards in almost every respect and it may be assumed that the diet of many other such wage earners can be little better than this.

Fat.—The figures for fat when compared with Western standards with the exception of the families are distinctly poorer than in the case of protein. The accepted standard is about 100 g. per day of which 50 g. is animal fat. All the institutions have a fat intake of not more than 50 g. of which in the case of the two orphanages only 3 g. to 12 g. is from animal sources. The Anglo-Indian school presents an apparently better standard with 34.6 g. of animal fat which, however, was derived almost entirely from suet or lard. It is probable that the fat requirements in the tropics are less than what is recommended in Europe but it would seem unlikely that health could be maintained on such a low proportion of animal fat. An adequate fat intake has yet to be found for tropical climates.

Minerals.—The two minerals investigated, namely calcium and phosphorus, would when compared to that recommended by Sherman appear to be distinctly below what is adequate, especially for children. Fortunately, the low calcium is associated with a correspondingly low phosphorus intake which probably means a more effective absorption of both than might otherwise be the case were one or other in excess. Sherman recommends about 1 g. calcium per day for a child, while the intake in the institutions varies from 0.23 g. to 0.40 g. per head. In the case of the Anglo-Indian school (0.2 g.) the Headmaster reported that some of the boys had been seen eating the plaster off the walls which is significant although this might be interpreted on other lines than natural instinct or craving. The calcium intake of the families is, however, quite within the accepted standard and it should be mentioned that adult Indians may obtain a certain amount of calcium from chewing betel leaves containing a lime paste flavoured with catechu. It is possible, however, in view of the ample sunlight and the even balance of calcium and phosphorus in the diet that the minimum requirements may be less than that recommended by Sherman. Again, however, it is difficult to conceive that it should be as low as 0.20 g. per day which in the case of the children is derived from sources other than milk. The higher calcium intake of the Muslim orphanage is due to the high consumption of atta (whole-wheat), while the low value in the Hindu one is due to the preponderance of rice in the diet. The low figure for the Anglo-Indian school was due to the fact that they consumed white bread and rice, both of which are poor in this mineral.

Distribution of calories.—The percentage distribution of calories between protein, fat and carbohydrate used to be and still is to a great extent a good guide as to the general balance of a diet. Within recent years, however, owing to the increasing significance and importance for good or ill of such foods as milk products, cereals, etc., analysis has taken on a less chemical aspect and tends to assess values in terms of food-stuffs belonging to a general class. Such a development is to be encouraged particularly when it is borne in mind that the terms protein, fat and carbohydrate, although accurate and valid from a chemical point of view, may have less significance in the total economy (metabolism in the wide sense) of the organism which is primarily concerned in the handling of food-stuffs or food as a whole. This is the more important in view of the fact that many cereals, although chemically almost identical, often appear to be associated with particular pathological conditions, such as maize with pellagra, rice with beri-beri, wheat with defects in the nervous system (Mellanby, 1934), cabbage with goitre—conditions which in the

human subject have not yet been exhaustively explained in terms of deficiency of vitamins alone.

In this investigation the distribution of the diet has been calculated in terms of the percentage distribution of calories between cereals and milk products respectively and the percentage of the food budget spent on milk products and vegetables. This monetary basis has been adopted with the latter in view of the fact that valuable vegetables in a diet may contribute but little to its calorie value. The full significance of vegetables will be much more easily assessed when figures for the vitamin content of the food-stuffs have been worked out.

In the U. S. A., Rose (quoted by Sherman, 1932) recommends a percentage distribution of calories from cereals and milk products of 24 per cent to 37 per cent and 22 per cent to 32 per cent respectively for children. For adults somewhat less milk and more cereal is accepted as a suitable figure for an adequate diet. The examination of the figures shows that they fall short considerably from those mentioned above, particularly in the case of the institutions. The maximum for dairy products is 3·57 per cent in the Anglo-Indian school and this fraction costs 11 per cent of the food budget.

On the other hand the cereal calories are high amounting to 82 per cent of the total in the Muslim orphanage. The percentage of the budget spent on fruits and vegetables in this institution is 9 per cent to 17 per cent, a reasonable figure, although at certain times of the year this will represent vegetables which require cooking as the cheaper fruits such as mangoes and oranges are not available. As regards the families the percentage of calories derived from dairy products ranges between 2·4 per cent and 29 per cent, average 12·99. In many cases the milk products have been the means whereby a rather mediocre diet has been largely improved. The percentage of calories derived from cereals on the other hand, although it varies widely, is on the average 54 per cent—less than that obtaining in the children's institutions but higher than Sherman's figures. The percentages of the calories derived from these two sources show up very clearly the way in which the Indian diets recorded here differ from those generally advocated in the West.

The solution of the problem of malnutrition in India in all probability will converge round a qualitative and quantitative balance of these two sources of food.

Cost of diet.—The cost of the family diets with two exceptions are extraordinarily low, ranging between annas 2·89 and 6·52 per M. V. per day. The cheapest diet of all was that of the sweeper, annas 2·21 per day. The average for all the families is only annas 6·65 per M. V. per day and taking everything into consideration they are by no means too bad. The figure, however, cannot be taken as the minimum cost of such a diet. In point of fact it might be improved with a reduction in cost particularly if carried out on a larger scale. The cost, however, in the institutions and in particular the hostel merits special attention. The average income of the men in the hostel is Rs. 40 per month. In general it may be assumed that each member is independent and has to feed and clothe himself and possibly remit money home. The standard of living is probably as good or even better than many students in Calcutta can afford to live at. The diet is possibly just adequate for a sedentary worker if the standard figure suggested by the League of Nations is

accepted. The other constituents speak for themselves and the cost is annas 4·13 per M. V. per day. The diet is qualitatively very little better than the two Indian orphanages which cost about annas 2·6 to 2·9 per M. V. per day, while it is distinctly poorer than that of the Anglo-Indian school which amounts to annas 4·13 per M. V. per day.

The poorest diet all over is that of the Oriya sweeper, the calories derived from milk products are less than 2 per cent of the total and cost 10 per cent of his budget. Taking the diets all over the best value for the money would appear to be that of the orphanages and school. The cost of the Anglo-Indian school is annas 4·13 per M. V. per day, almost 33 per cent more than the other two. As far as India is concerned a diet such as this school provides could be procured at a slightly lesser cost by substituting atta for white flour which is cheap and incidentally would increase the Ca and P content. In addition this school used small quantities of European manufactured products such as cocoa and jam which might be replaced by some local sweatmeats at a lower cost. An average cost of about annas 4 per M. V. per day is a likely figure for such a diet, which apart from theoretical reasons, appears to work reasonably well as regards the health and growth of the children. In all these institutions, however, the diets might be passed taking Western standards into consideration, with a reasonable assurance of adequacy if one pint of milk per head were added. This would increase at the cost by about annas 1·5 per day bringing the total figure per M. V. per day for the diet to between annas 4·4 and 5·6. As far as the inhabitants of Calcutta are concerned and with present prices this would entail an expenditure of Rs. 7 to Rs. 10 per month per head which is undoubtedly out of the question for the masses and even for a large number of wage earners of Rs. 40 to Rs. 80 per month with families to keep.

DISCUSSION.

There can scarcely be any question that all the diets analysed fall below Western standard in most respects although in varying degree. In short they are all-round deficient diets. At the same time analysis shows this deficiency to have a bias in certain directions, namely animal protein, animal fat, dairy products, calcium and excess of cereal. Unfortunately these deficiencies are more marked in the children's institutions and the same is likely to hold good for children of parents who would belong to the class living in the above-mentioned hostel. Assuming that the Western standard is not rigidly applicable in India and this is probable in the case of fat, the degree of divergence between the figures collected here and the accepted standard is too great to be dismissed as falling within the range of what constitutes a good diet or what the human species can adapt itself to. It would appear to be justifiable to recommend a reduction in the quantity of cereal, an increase in the proportion of atta and an increase of milk products. At the present time the simplest expedient would be an increase in the consumption of milk or milk products. In a population where meat is largely forbidden and fish not everywhere obtainable, milk, which is permitted by nearly all, would supplement most of the deficiencies, covering in addition those properties present in atta the introduction of which into rice-eating areas though relatively cheap would be by no means easy.

The relation between deficient diet and disease is yet to be worked out in India. It appears to the writers that more accurate data on food consumptions and detailed

analysis of disease covering infant and maternal mortality and morbidity, diseases of children and certain conditions of middle age will have to be collected before any real correlation can be made.

It is unlikely that any one deficiency, such as a vitamin or mineral, will be the cause of any condition. The peculiar qualities of certain different types of food, chemically similar, such as maize or atta, may, with or without a vitamin deficiency, appear to condition certain departure from health. The possibility of certain deleterious properties of foods, such as the toxamins, should not, however, be sufficient reason to discard them for consumption, other factors being taken into consideration.

CONCLUSIONS.

(1) The diets analysed in this survey are poor in total and animal protein, total and animal fat, calcium and to a lesser extent phosphorus. They contain too low a percentage of dairy products and an excess of cereal.

(2) The minimum cost in Calcutta at current prices to-day of a diet which approaches to within a reasonable degree the Western standards is somewhere in the region of annas 4.4 to 5.6 per man value per day. Under present conditions this is beyond the means of most.

(3) As an expedient to improve the diets an increase in the consumption of milk products would be the easiest to introduce and in the long run the cheapest.

(4) An increase in the consumption of atta in the rice-eating districts is recommended.

(5) The collection of more data on food consumption in India is strongly urged in order to obtain suitable dietary standards.

(6) The association of diet with disease in India can only be determined by a careful diet survey in relation to clinical observations within the same area.

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THE BASAL METABOLISM OF YOUNG MEN AT HYDERABAD (DECCAN) WITH A STUDY OF THEIR PHYSICAL CHARACTERS*.

BY

S. A. RAHMAN.

*[From the Department of Physiology, Osmania University Medical College,
Hyderabad (Deccan).]*

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INTRODUCTION.

EVER since the remarkable piece of work by A. Ozorio de Almeida (1921) in Brazil, the interest of the workers in metabolism has been aroused as to the influence of the racial and the climatic factors in modifying basal metabolism. de Almeida, who studied the basal metabolism of 10 white men and 10 negro labourers using a Tissot gasometer, discovered that the metabolism of the whites was considerably lower than the generally accepted standards for the United States and that the metabolism of the negroes, though higher than that of the whites, was still much below the figures obtained in the temperate zones.

These findings of de Almeida have not been substantiated by many other workers. Prior to de Almeida's work, Eijkmann (1896, 1921), using a Zuntz-Geppert apparatus, studied 12 Malays and 11 Europeans living in Batavia and found no difference in the metabolism of the Malays, Europeans in Batavia and Europeans studied in Germany by Geppert, Loewy and Magnus Levy. Takahira (1925) studied the basal metabolism of 120 Japanese men and women and came to the conclusion that there was no significant difference in metabolism between the Japanese and the Europeans.

On the other hand Montoro (1921, 1922) in Havana obtained results similar to those of de Almeida. The results obtained by Knipping (1923) and confirmed by Fleming (1925) show that prolonged residence in the tropics tends to lower the basal metabolism.

Though the evidence is in favour of the inference that residence in the tropics tends to cause lowering of the basal metabolism, recent investigations suggest that

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there is also a racial factor involved. MacLeod, Crofts and Benedict (1925) studied 7 Chinese and 2 Japanese women who had been students in American colleges for at least fifteen months under the same climatic, dietetic and other conditions as Western women. Their metabolism averaged 10·4 per cent below the Harris-Benedict standards (1919) for women and 10·2 per cent below the Aub-DuBois standards (1917). The authors have found both these standards about 5 per cent too high for American college women. But even then this leaves the Oriental women about 5 per cent below the American standards. This is in spite of the fact that for over a year the environmental conditions were exactly the same in both the cases. Williams and Benedict (1928) and Shattuck and Benedict (1931) carried out a series of studies on the Maya Indians in Yucatan in connection with the comprehensive programme of the Carnegie Institution of Washington for investigating racial metabolism. They used a field respiration apparatus. These investigations brought out the extraordinary fact that the average metabolism of the Mayas was somewhat above the basal metabolism of the North American whites. This is the first report in which a tropical or sub-tropical race has shown metabolism higher than that of the American or the European standards.

Again, in the third expedition to Yucatan, Steggerda and Benedict (1932) re-investigated the metabolism in the Maya Indians. They confirmed the results obtained previously that the male Mayas have a high basal metabolism and a low pulse rate. A group of 30 male Mayas were found to have heat production on the average 8 per cent above the prediction standards for the whites. They definitely concluded that this high metabolism was largely due to the racial factor involved. This will be discussed later along with the other factors which have been supposed to influence metabolism.

Basal metabolism observations in India.—In India very few observations have so far been made. Mukherjee (1926) reported the results of observation on 15 male Bengalis between the ages of 22 and 27 years. The measures were carried out with the Douglas bag and the Haldane's gas analysis apparatus when the subject had been without food for 16 to 18 hours at a room temperature averaging 27°C. The metabolism on the average was 9 per cent below the Sanborn standards (1933). In a second paper Mukherjee and Gupta (1931) reported a new series of healthy Bengali men of ages from 20 to 29 years whose metabolism averaged 13·3 per cent below the DuBois standards with individual variations of from -0·3 to -31·2 per cent. Sokhey (1927), using a Collin's chain compensated gasometer (a modification of Tissot's gasometer) with the Haldane's air analysis apparatus (Henderson's modification), measured the basal metabolism in 21 male medical students in Bombay between 19 and 30 years of age. He found that 15 of his subjects showed a basal metabolism 10 to 23 per cent lower than the DuBois standards. The details of the experiments are not given.

Recently, Mason and Benedict (1931) have investigated the basal metabolism of 54 women (Tamils, Malayalis, Telugus, Coorgs and Kanarese) in South India of ages ranging between 17 and 31 years by means of Benedict's portable apparatus. They obtained results 16·9 per cent lower than the Harris-Benedict standards and 17·2 per cent lower than the Aub-DuBois standards and conclude that there is a definite racial factor.

All these investigations were carried out at seaside places. So far as I am aware, with the exceptions of the tests done at Lucknow which are discussed elsewhere, basal metabolism investigations have not been reported from the interior of India. The present investigations were undertaken with a view to study the physical characters of young men in Hyderabad which is situated at a higher level (nearly 1,700 feet above sea-level) and has a drier climate than those places in India whence the basal metabolism results have so far been received.

TECHNIQUE.

The apparatus used was the Sanborn Motor-Grafic Metabolism Tester, manufactured by the Sanborn Company, Cambridge, Mass. It is a spirometer type of respiration apparatus based on the same principle as the Benedict-Roth apparatus. It consists of an inverted bell supported by a counter-weight and floating on water. The bell is filled with medicinally pure oxygen from a small cylinder which is conveniently fixed to the trolley carrying the apparatus. The subject breathes into and out of the bell through a rubber mouthpiece, the nose having been closed by means of a nose-clip. The rubber mouthpiece is connected to an oxygen control valve. When this valve is open, the subject breathes the outside air; when it is closed, the subject is connected to the breathing circuit of the apparatus. The expired air is led through a vessel containing sansoline (supplied by the Sanborn Company) which absorbs the carbon dioxide and the water vapour and the subject breathes in almost pure oxygen. The movements of the bell which correspond to the respiratory movements of the subject are registered on a chart applied to a revolving cylinder which moves by means of clockwork.

The apparatus indicates only the oxygen consumption of the subject and in this respect the results are not so accurate as those obtained by the Douglas bag or the Tissot methods, which measure both the oxygen consumption and the carbon dioxide production of the body from which the actual respiratory quotient may be calculated. On the other hand, a great advantage of the apparatus is that the results are graphic and permanent (Beaumont and Dodds, 1935). The rate of the absorption of oxygen is indicated by the rate of decline in the level of the writing-ink pen which is attached to the spirometer. This method offers a means of checking up leaks that may develop in the apparatus during the test; a very rapid fall in the level of the pen being caused by a leak. Another great advantage of this method is that it registers the mode of breathing. It is reported by Earle and Goodall, and Hannon and Lyman (quoted by King, 1924) that only subjects in whom breathing is regular give satisfactory results by the close system, and other results are readily weeded out by the record of respirations upon a kymograph. This is certainly true to some extent. I have found that the most constant and accurate results are obtained when the breathing is uniform and regular. If the breathing is irregular, the results obtained are almost invariably too high.

Another advantage of the apparatus is the convenience in calculation; the oxygen consumption per minute is directly read off from the chart, thus eliminating the personal factor in the measurements of the gases.

The apparatus can be used with the motor blower or without it. In the latter case rubber valves are substituted for the motor blower. The motor blower was used, however, only in a few cases. It was found that, with the motor blower in

TABLE I.

Subject number.	Name.	Age, years.	Body-weight (without clothes), kg.	Height, cm.	Sitting height, cm.	Pelidisi.
1	A. Ahmed ..	24	51.7	174.7	90.6	89
2	A. Manan ..	20	58.5	171.2	87.3	96
3	S. A. Khan ..	23	50.8	170.7	88.9	90
4	L. A. Khan ..	23	56.2	170.7	88.9	93
5	S. A. Husain ..	23	47.2	167.9	88.1	88
6	M. Y. Zubairi ..	22	55.8	171.5	90.2	91
7	M. Imamuddin ..	21	45.4	166.8	87.6	88
8	T. N. Reddi ..	20	55.8	174.0	89.4	92
9	S. M. Ali ..	22	55.5	162.0	..	..
10	S. A. Husain ..	27	59.0	166.4	84.6	99
11	A. Mohiuddin ..	19	55.0	175.5	89.7	91
12	R. R. Saksena ..	22	48.5	168.6	85.8	92
13	H. A. Ansari ..	21	46.5	168.3	86.9	91
14	M. Sharluddin ..	19	43.2	166.0	87.6	86
15	M. Musiquddin ..	20	45.4	168.6	87.9	87
16	Jagmohan ..	20	52.0	166.6	85.8	94
17	Bhushnam ..	21	49.0	160.2	86.4	91
18	I. Rahman ..	20	56.5	178.6	91.4	90
19	Q. S. A. Rashid ..	22	52.5	170.7	90.9	89
20	K. S. Manvikar ..	22	71.3	177.7	93.9	95
21	R. Karan ..	22	53.0	177.0	..	..
22	Q. Ali ..	20	55.5	174.0	90.0	91
23	M. R. Beg ..	20	63.0	174.5	94.2	98
24	I. Ahmed ..	21	57.0	164.0	86.6	96
25	R. N. Abhyankar ..	32	54.0	165.1	87.9	93
26	P. Shankarya ..	22	57.2	162.2	83.8	99
27	X. Ramiah ..	20	46.5	164.5	86.4	90
28	S. Ramiah ..	19	41.5	168.0	86.4	86
29	R. B. Phatkay ..	20	56.2	172.0	90.3	91
30	I. G. Gadray ..	28	60.2	159.5	85.3	99
31	Ranade ..	31	68.2	162.5	87.6	100
32	V. G. Gadray ..	23	68.2	167.0	89.1	99
AVERAGES ..		22	54.2	169.3	88.3	92 ± 4

Sitting height.—The sitting height was actually measured in every case with all the precautions emphasized by Dreyer and Hanson (1921). As mentioned by Pirquet (1922), the sitting height thus measured equals, in Westerners at least, one-half of the total height plus 5 cm. Table II shows the actual sitting heights obtained in the case of my subjects as compared with the sitting heights calculated according to the formula, $h/2$ plus 5 cm. where h stands for the total height. It is seen that the actual sitting height in most of the cases is less than the calculated sitting height. The average sitting height works out as 1.2 cm. less than the calculated average. From this it is seen that the average young Indian has a stem height about 1.2 cm. less, and therefore correspondingly longer legs, than the average Westerner. Even greater difference in sitting heights was found by Mason and Benedict (1931) in the case of their women subjects. They found that with the Tamils one-half of the average total height was 77 cm. and the actually measured sitting height was, on the average, only a little over 1 cm. greater than this. Much the same picture was shown by their other groups.

TABLE II.

Subject No.	Height, em.	SITTING HEIGHT, CM.			BODY-WEIGHT (WITHOUT CLOTHES), LB.			DEVIATION FROM NORMAL.		
		Actual.	Calculated.	Difference.	Actual.	American standards.	Difference.	Harris-Benedict, per cent.	Aub. DuBois, per cent.	Sanborn, per cent.
1	174.7	90.6	92.3	1.7	114	146	32	14.5	17.1	—
2	171.2	87.3	90.6	3.3	129	136	7	6.7	7.9	—
3	170.7	88.9	90.3	1.4	112	137	25	2.7	0	+
4	170.7	88.9	90.3	1.4	124	138	14	5	6.8	—
5	167.9	88.1	88.9	0.8	104	134	30	1	1.2	—
6	172.7	90.2	91.3	1.1	123	140	17	2.7	0.4	+
7	166.8	87.6	88.2	0.6	100	120.5	20.5	17.4	19	+
8	174.0	89.4	92.0	2.6	123	140.0	17.0	10	11	—
9	162.0	87.0	86.0	1.0	122	125	3.0	9	16	—
10	166.4	84.6	88.2	3.6	130	134.5	4.5	14	10	—
11	175.5	89.7	92.7	3.0	119	140	21.0	0.8	4.3	—
12	168.6	85.8	89.3	3.5	107	134	27.0	6	7.8	—
13	168.3	86.9	89.1	2.2	102	131	29.0	16	17.7	—
14	166.0	87.6	88.0	0.4	95.5	136	40.5	8	2.3	+
15	168.6	87.9	89.3	1.4	100	132	32.0	1	4.5	+
16	166.6	85.8	88.3	2.5	114	128	14.0	3	2.2	+
17	160.2	86.4	85.1	1.3	108	119.5	11.5	11	12.5	—
18	178.6	91.4	94.3	2.9	124	148.5	24.5	8	8.8	—
19	170.7	90.9	90.3	0.6	115.5	136	20.5	1	4.15	—
20	177.7	93.9	93.8	0.1	157	148	9.0	10.5	10.5	—
21	177.0	87.0	93.5	6.5	117	144	27.0	11.5	14.65	—
22	174.0	90.0	92.0	2.0	122.5	140	17.5	9	10	—
23	174.5	94.2	92.2	2.0	139	142	3.0	8	7.6	—
24	164.0	86.6	87.0	0.4	125	125	0	17	17.2	—
25	165.1	87.9	87.5	0.4	119	135	16.0	10	16.4	—
26	162.2	83.8	86.1	2.3	125.4	125	0.4	4.25	5.15	—
27	164.5	86.4	87.2	0.8	102	126	20.0	0.6	1.5	—
28	168.0	86.4	89.0	2.6	91	130	39.0	11	14.6	+
29	172.0	90.3	91.0	0.7	123	137	14.0	12	13.4	—
30	159.5	85.3	84.7	0.6	132	125	7.0	9.2	11.9	—
31	162.5	87.6	86.2	1.4	150	130	20.0	6.5	7.8	—
32	167.0	89.1	88.5	0.6	150	133	17.0	6	4.9	—
AVERAGES	..	..	..	1.2	119.3	134.3	15.0	6.8	8.7	7.4

Pelidisi.—The usual clinical classification of well-nourished, moderately well-nourished, poorly nourished, etc., is not precise and therefore not satisfactory as an indication of the state of nutrition of an individual. As the basal metabolism is affected by the nutritional state of the individual, it is essential for the study of the normal subject to have a precise knowledge of his state of nutrition. The Nutrition Laboratory of the Carnegie Institution of Washington has found the pelidisi of Pirquet of great advantage over the ordinary, rather gross, clinical indications (Shattuck and Benedict, *loc. cit.*).

Pelidisi is the relationship between the weight of the individual and his sitting height. This method of fixing the state of nutrition of an individual was first originated by the late Professor Clemens Pirquet (*loc. cit.*) of Vienna and is known as the Pirquet index.

It is expressed by the formula

$$\sqrt[3]{\frac{10 \times \text{weight in g.}}{\text{Sitting height in cm.}}}$$

and the result is multiplied by 100.

In the original preparation of the material, Pirquet assumed that for small children a pelidisi of 100 represented the ideal. It has since been found that for adults a value somewhat less than this, probably nearer 97 or 98, represents the normal state of nutrition. It has been found that with Westerners a pelidisi around 90 represents a distinctly low state of nutrition and that a pelidisi over 100 represents a fat person.

Table I shows the pelidisi in the case of my subjects as it has been calculated from the sitting height actually obtained. The majority of the results are over 90, the minimum being 86 in the subjects Nos. 14 and 28, the maximum being 100 in the subject No. 31. The average for 30, out of the 32 subjects, works out as 92 ± 4 .

It is, therefore, clear that the pelidisi as obtained in these subjects is less than that which is accepted as representing the normal state of nutrition in the Westerner. Does this denote that these subjects were under-nourished? I do not think so. Exceptions might be made in the case of subjects Nos. 14 and 28 who were unusually thin. The rest were certainly in good health and were getting good food. It would probably be more correct to infer from the results that a pelidisi of about 92 indicates the normal state of nutrition in young Indians. That the pelidisi showing the normal state of nutrition may differ in different races is suggested by the measurements in the Chinese. Stevenson (1928), after examining over three thousand of the Chinese, came to the conclusion that the Chinese approaches the pelidisi of 90 as the basic standard. Benedict and Meyer (1933) found that the average pelidisi of their 18 American-born Chinese girls (12 to 22 years of age) was 92.

Blood pressure.—The blood pressures were taken after the basal metabolism tests were over and before the subject had partaken of any food. The instrument employed was of Riva Rocci type fitted with mercury monometer, the zero point of which was carefully adjusted. The elastic cuff was about 12 cm. broad. This is assumed to give an error of from plus 7 to plus 9 per cent compared with the pressures obtained directly from the artery (Norris, Bazett and McMillan, 1928). The auscultatory method was employed. The subject was made to sit on a stool with the left hand, which was used for examination, resting relaxed on a table in front,

palm upwards. In this posture the brachial artery was level with the heart. Readings were taken during release of pressure. Readings of the systolic pressure was taken at the point at which the first clear thump was heard below the cuff (first phase), the stethoscope being snugly applied to the artery. The diastolic pressure was used at the so-called fourth phase, i.e., at the point where the second clear thump becomes suddenly muffled. Several consecutive readings were taken to ensure correctness of results, the pressure between the readings being allowed to fall to zero and sufficient time permitted to elapse for the venous pressure to fall to normal level. The psychical element of interference in the case of these subjects was negligible and did not interfere in obtaining correct results.

The blood-pressure estimates show that the readings are invariably lower than what has been taken as normal for Westerners. The average systolic pressure obtained was 100.4 ± 11 mm. of mercury, the maximum being 125 mm. in subject No. 4 and the minimum being 75 mm. in subject No. 22 (Table III). The normal systolic pressure in Westerners as studied by Melvin and Murray (1914) was 112 mm. for young men between the ages of 20 and 29 years. Fisher (quoted by Norris *et al.*, *loc. cit.*) found that, in 64,574 cases studied by him, the average auscultatory systolic pressures was 123.2 mm. ranging between 116 mm. at 16 years and 136 mm. at 65 years. More important are the results obtained by Alvarez (1920, 1923) who, unlike Fisher, did not base his results on insurance statistics but studied students in a State-controlled University. He found that 45 per cent of the male students had pressures exceeding 130 mm. which he considered to be abnormal. According to Alvarez, the normal upper limit of the systolic blood pressure for healthy young men is 130 mm.

The lower limit of the normal systolic blood pressure, according to Norris, Bazett and McMillan (*loc. cit.*), is usually 110 mm., and when this pressure goes below 105 few can be on their feet regularly. It is evident that these figures cannot be applied to Indians.

Diastolic pressure.—The average diastolic pressure of 71.8 ± 5 mm. in my subjects (Table III) is in keeping with the estimate of Woley (1910) who felt that it should be 70 per cent of the systolic pressure. Fisher, in the cases above referred to, found that the diastolic pressure had averaged 80 mm.

Pulse pressure.—Fisher, basing his results on the insurance statistics, above referred to, found that the average pulse pressure for all ages was 43.2 mm. This figure tallies closely with that of Symond (1923) who found the average pulse pressure for all builds and all ages to be 44.1 mm. Norris, Bazett and McMillan (*loc. cit.*) place the limits of normal pulse pressure between 30 mm. and 50 mm. and regard a pulse pressure persistently as low as 20 mm. or as high as 60 mm. as pathological.

The average pulse pressure in my subjects is 28.6 ± 7.2 mm. which is lower than what Norris *et al.* (*loc. cit.*) regard as minimum in the normal range. The minimum pulse pressure obtained is 19 mm. in the case of subjects Nos. 24 and 28, and the maximum is 35.7 mm. in the subjects Nos. 3 and 6. These pulse pressures, though very low compared to the Western standards, are, however, quite in proportion to the systolic and the diastolic pressures obtained in the subjects.

TABLE III.

Subject number.	Date.	Mouth temperature, °F.	BLOOD PRESSURE.				PULSE RATE.			RESPIRATION RATE.	
			Systolic.	Average systolic.	Diastolic.	Average diastolic.	Average pulse pressure.	Before start of experiment.	During experiment.	Before experiment.	During experiment.
1	8-9-33	98.2	92	94.5	64	63	26.5	48	54	21	18
1	14-10-33	98.1	97		72			55	53	15	14
2	8-9-33	98.5	100	95.5	68	65.5	30.0	56	49	15	28
2	8-4-34	98.5	91		63			67	73	22	21
3	8-9-33	99.0	122		80			86	82	19	12
3	18-10-33	98.8	112	113	80	77.3	35.7	78	77	15	15
3	22-8-34	98.4	105		70			67	60	18	15
4	27-9-33	98.2	125	125.0	85	85.0	40.0	66	70	..	..
5	27-9-33	98.0	110	110	65	65	45	73	68	..	..
6	27-9-33	98.4	123		75			66	66	16	12
6	28-9-33	98.0	108	114.2	75	78.5	35.7	63	61	16	12
6	8-10-33	98.0	110		92			61	61	11	12
6	18-10-33	97.9	116		82			61	61	13	10
7	28-9-33	98.0	108	108	75	75	33	63	61	16	14
8	28-9-33	97.6	115	115	75	75	40	62	62	19	14
9	27-9-34	97.7	95	95	70	70	25	53	53	22	31
11	8-10-33	98.4	120		100			..	..	13	16
11	19-10-33	97.5	108	111	85	97	24	72	75	14	12
11	8-4-34	98.2	105		76			78	77	17	15
12	9-10-33	98.5	103								

13	12-10-33	97-5	102	102	102	72	72	72	30	65	60	20	17
14	12-10-33	99-4	98	98	98	68	68	68	30	93	93	22	21
15	14-10-33	98-7	110	110	110	72	72	72	38	78	82	19	17
16	15-10-33	98-5	111	111	111	70	70	70	35-5	54	54	15	15
16	31-7-34	98-0	95	95	95	65	65	65	35-5	54	53	18	15
16	1-8-34	98-0	..	..	..	..	..	..	35-5	53	51	20	16
17	21-10-33	98-0	95	95	95	65	65	65	30	58	58	20	14
18	18-10-33	98-4	85	85	85	64	64	64	21	60	62	20	21
19	24-3-34	98-4	97	97	97	73	73	73	24	61	60	17	11
20	25-3-34	98-0	98	98	98	67	67	67	31	57	57	17	14
21	31-7-34	98-4	100	100	100	75	75	75	25	60	61	18	15
21	1-8-34	98-2	..	..	..	..	..	..	25	61	60	18	17
22	27-9-34	98-2	75	75	75	60	60	60	15	51	53	21	21
23	28-8-34	98-2	105	105	105	75	75	75	30	67	65	15	12
24	28-8-34	98-4	97	97	97	78	78	78	19	65	60	20	19
26	22-8-34	98-0	85	85	85	65	65	65	20	52	51	19	14
26	23-8-34	97-6	..	..	..	..	..	..	20	47	44	17	16
27	9-10-34	99-0	84	84	84	64	64	64	20	79	71	20	17
28	9-10-34	99-0	89	89	89	70	70	70	19	75	74	20	18
29	12-10-34	98-4	98	98	98	73	73	73	25	71	67	21	20
30	12-10-34	98-2	112	112	112	78	78	78	34	85	81	22	13
31	27-10-34	97-6	95	95	95	75	75	75	20	56	57	15	9
32	27-10-34	98-4	105	105	105	75	75	75	30	65	68	23	18
AVERAGES ..		98-3	..	100-4	100-4	..	71-8	71-8	28-6	65	64	18-7	16-4
				± 11	± 11		± 5	± 5	± 7-2				

Previous observers in the tropical and sub-tropical countries have also noted low blood pressure in the inhabitants. McCay (1907) investigated the blood pressure in a large number of the Bengali students. The readings for the systolic blood pressure were taken at the disappearance of the pulse at the wrist. The pressure varied between 83 mm. and 118 mm., the average being slightly over 100 mm. (sitting position, arm level with heart). The observations on the Philippines by Musgrave and Sisson (1910) also indicate a lower systolic level. Cadbury (1922) studied the Chinese students and found their systolic blood pressure from 20 mm. to 30 mm. and diastolic pressure from 10 mm. to 20 mm. lower than the normal one finds in the inhabitants of North America and Europe.

Recent investigations in India give further evidence of low blood pressure in the Indians. Mukherjee and Gupta (*loc. cit.*) recorded the blood pressure of 12 Bengali students and found the average systolic to be 108 mm., average diastolic 78 mm. and the pulse pressure 30 mm. The *Indian Medical Record* (1931) gives the average systolic pressure of healthy Punjabees, between the ages of 21 and 25, as 114 mm. and the diastolic pressure as 74 mm. Stock and Karns (1924) examined 14 Indian students resident in London along with 103 British male students. They found a systolic average of 118.7 and a diastolic of 80.3, while the corresponding figures for the British group were 130.3 and 84.8 respectively. More recently, Subba Reddy (1933) has investigated the blood pressure in 112 male students of Vizagapatam Medical College between the ages of 18 and 27 years. His results show that 41.07 per cent of his subjects have the systolic pressure between 111 mm. and 120 mm., and 23.21 per cent between 121 mm. and 130 mm. The average systolic pressure was 116.8 mm., the average diastolic 76.9 mm., and the average pulse pressure 39.9 mm. These results are higher than those obtained by Cadbury in the Chinese students and higher than the results obtained in the case of my subjects. The high results obtained in Vizagapatam students may partly be due to the fact that the investigations were made in the afternoon presumably after the midday meal when the blood pressure may be expected to be somewhat higher. Even then the results obtained at Vizagapatam are lower than those given for Westerners by Fisher and Alvarez.

It is possible that the warmer climate of the tropical and sub-tropical countries lowers the blood pressure by causing the dilatation of the skin capillaries. That environmental conditions are an important factor in determining blood pressure is brought out by the studies of Tung (1928) on the Chinese. Blood pressure was examined in the case of 30 Chinese during their stay in America and also after their return to Peking. The average systolic pressure in America was found to be 113 mm. and in China 102 mm. The average diastolic pressure in America was 72, and it was 64 in Peking. Tung also reports that there is a distinct tendency for the blood pressures of Westerners resident in China to approach that of the Chinese. It therefore seems, as Tung suggests, that climatic differences are the main determining factors of blood pressure.

Mouth temperature.—This ranged between 97.5°F. and 99.0°F., the average working out as 98.3°F. (Table III). The mouth temperature was taken before the basal metabolism test was started, the subject lying on the metabolism cot. The thermometer was kept in the mouth for at least two minutes.

Pulse rate.—Column 9 of Table III gives the pulse rate taken just before starting the basal metabolism tests. This is compared with the rate of the pulse taken while the tests were in progress. It is seen that the former is slightly higher than the latter.

The pulse rate, taken before starting the metabolism tests, ranged between 53 in the case of subjects Nos. 9 and 16, and 86 in the case of subject No. 3. The average for 30 subjects works out as 65.

The data on the basal pulse rate are numerous in the case of Western subjects. Körösy (1910) counted the pulse rate of 255 soldiers, between the ages of 20 and 24 years, early in the morning before they had arisen from their beds and partaken of any food. He found the average rate of 64.2 with the maximum of 108 and the minimum of 42. Sutliff and Holt (1925) established for 146 adult men an average pulse rate of 62. Harris and Benedict (1919) (quoted by Boas and Goldschmidt, 1932) found the average basal pulse rate in the case of 121 adult males as 61.26 ± 6.73 . Boas and Goldschmidt (*loc. cit.*) found in 51 males of the average age of 28.1 years, the basal pulse rate of 61.4 ± 8.22 . They state that the basal pulse rate is lower and less variable than the heart rate measured under less standardized conditions, because many extra-cardiac factors that affect the heart rate are eliminated in the post-absorptive resting state.

It is seen that the pulse rate in the case of my subjects corresponds closely with the results obtained in the Westerners.

Mason (Mason and Benedict, 1931) also found that the average pulse rate in the case of the South Indian women corresponded closely with the average pulse rate in 90 American women studied by Harris and Benedict (*loc. cit.*).

That the pulse rate may differ with certain races is suggested by the observations of Steggerda and Benedict (*loc. cit.*) on the Mayas. They found in 30 male Mayas an average pulse rate of 52, with the minimum rate of 34—figures considerably lower than the average established in other peoples.

Respiration rate.—The respiration rate was counted before commencing the metabolism tests, the subject lying on the metabolism cot. Precautions were taken not to attract the subject's attention to his respirations. The respirations were counted for fully one minute. The lowest rate was in the subject No. 6, being 11, 13, 16 and 16 on the four different dates. The highest rate was 23 in the subject No. 32. The average for all the subjects works out as 18.7 per minute. There seems to be little difference between the respiration rates as obtained in these subjects and those described for Westerners. M'Kendrick (1889) mentions that in health there are usually 15 respirations per minute in the adult. Quetelet (quoted by M'Kendrick, *loc. cit.*) gives a table indicating the maximum respiration rate between the ages of 20 and 25 years as 24 per minute, the minimum 14 per minute and the average 18 per minute. According to Burton-Opitz (1921), the average rate of respiration between these ages is 18.7, exactly the same as has been obtained in the case of my subjects. Halliburton and McDowall (1930) give the rate of respiration in a healthy adult person as ranging from 14 to 18 per minute. Thus it would seem that, considering the warmer climate and the lower barometric pressure (about 720 mm. Hg.), the respiration rate in these subjects is not any higher than what has been recorded for Westerners.

Table III gives also the respiration rates during the periods in which the metabolism tests were carried out, i.e., the periods in which the subjects were connected with the metabolism apparatus. It is seen that in such a state the respiration rate is almost invariably lower, the average working out as 16.4. This accords with the experience of the Nutrition Laboratory of the Carnegie Institution of Washington that, not invariably but in general, there is a tendency for the respiration rate to become somewhat slower when any breathing appliance is attached (Shattuck and Benedict, *loc. cit.*; Mason and Benedict, 1931).

The respiration rates so obtained are not far from those found in the case of the Mayas by Shattuck and Benedict (*loc. cit.*) under similar conditions and which were not considered by them as far from the normal.

Surface area.—The necessity to obtain the surface area of the body has arisen in order to find the basal metabolism. A commonly accepted standard of the basal metabolism is the amount of heat produced per square metre of the body surface per hour. The surface area, however, was not actually measured but was obtained by the help of the excellent chart prepared by DuBois and DuBois (1916) (quoted by DuBois, 1927) and based on his height-weight formula.

In the case of the Chinese adults, it has been shown by Waddell, Han and Ch'en (1928) that the DuBois height-weight formula for the estimation of surface area is applicable with as much accuracy as in the case of the American subjects. Whether it can be applied with as much accuracy in the case of the Indians with smaller stem height is problematic.

Oxygen consumption.—This was measured with all possible care. All possible attention was given to guard against leakage. The mouthpiece gave no trouble, but the nose-clip required particular and, in some cases, constant attention. It had to be applied tight enough to prevent any leakage through the nose and at the same time not so tight as to be uncomfortable to the subject. The leak-tester was frequently used both before starting the chart to run to see if the nose-clip was properly applied or not, and also during the time when the chart was running and the respiration recorded. Occasionally it was found that the nose-clip seemed to have had been applied properly before starting a test, the leak-tester indicating no leakage; however, when the actual test was going on slight leakage was discovered during some period of the test. Such tests were obviously incorrect and were discarded. The leak-tester consisted of a brightly polished white metal piece. This was cooled by keeping it in cold water and, before use, was wiped clean with a piece of white muslin. During the monsoon season it was found that the use of ice-cold water to cool the leak-tester was not proper. This made the leak-tester too cold, so that when it was removed from the ice-cold water and wiped clean for use, it was found that a thin film of moisture from the atmosphere, which was nearly saturated, was deposited on the surface and made the leak-tester unsuitable for work. Water a few degrees colder than the tap-water was best for the purpose.

Usually two tests were made on a subject on any particular day. The oxygen consumption per minute was read off from the chart. This is shown in detail in column 6 of Table IV. It is seen that the results obtained of the two consecutive tests are usually within 5 per cent of each other. Column 7 of the same table gives the average of two or more tests done on the same day. Occasionally a test had to be discarded when a leak was discovered or when the breathing was very

TABLE IV.

Subject number.	Date.	Weight (without clothes), kg.	Height, cm.	Surface area, sq. m.	Oxygen consumption per minute, c.c.	Average oxygen consump- tion per minute, c.c.	HEAT PRODUCTION PER HOUR CAL.		DEVIATION FROM NORMAL.		
							Total.	Per sq. m.	Harris- Benedict.	Abb- DuBois.	Sanborn.
1	8-9-33	51.7	174.7	1.63	194	194	56.2	34.5	- 9	- 13	- 11
1	14-10-33	52.0	174.7	1.63	180, 177	178.5	51.65	31.3	- 20	- 21.2	- 18.5
2	8-9-33	58.5	171.2	1.69	207	207	59.9	35.5	- 9	- 10	- 10
2	8-4-34	55.4	171.2	1.65	206.5, 217.5	212	61.4	37.2	- 4.5	- 5.8	- 5.5
3	8-9-33	51.0	170.7	1.59	226	226	65.4	41.2	+ 7	+ 4.3	+ 5.0
3	18-10-33	51.0	170.7	1.59	205.0, 210.5	207.7	60.1	37.8	- 1	- 4.3	- 3.5
3	22-8-34	49.5	173.0	1.58	215.0, 216.0	215.5	62.35	39.5	+ 2	± 0	+ 0.2
4	27-9-33	56.2	170.7	1.66	211	211.0	61.1	36.8	- 5	- 6.8	- 5
5	27-9-33	47.2	167.9	1.52	205	205.0	59.3	39.5	- 1	- 1.2	- 1
6	27-9-33	56.0	172.5	1.67	245	245.0	70.9	42.5	+ 10	+ 7.6	+ 10

TABLE IV—*concl'd.*

No.	Date.	Weight (kg.)	Height, cm.	Surface area, sq. m.	Oxygen consumption per minute, c.c.	Average oxygen consump- tion per minute, c.c.	HEAT PRODUCTION PER HOUR CAL.		DEVIATION FROM NORMAL.		
							Total.	Per sq. m.	Harris- Benedict.	Aub. DuBois.	Sanborn.
6	8-10-33	55.8	171.5	1.66	212	212.0	61.4	36.98	- 4	- 6.4	- 4
6	18-10-33	55.8	171.5	1.66	233.0, 220.0	226.5	65.52	39.5	+ 2	± 0	+ 3.5
7	28-9-33	45.4	166.8	1.48	163.5	163.5	47.35	31.99	- 17.4	- 19	- 18
8	28-9-33	55.8	174.0	1.67	203.0	203.0	58.8	35.2	- 19	- 11	- 12
9	27-9-34	55.5	162.0	1.59	197.5, 193.0	195.2	56.5	35.5	- 9	- 10	- 8.7
10	17-8-33	59.0	166.4	1.67	190.0	190.0	55.0	32.93	- 14	- 16	- 14
11	19-10-33	55.0	175.0	1.67	205.0, 207.0, 214.0	208.7	60.4	36.2	- 8	- 11.7	- 9.3
11	8-10-33	54.0	175.5	1.66	224.5, 231.5	228.0	66.0	39.8	+ 2	- 3.0	- 1.5
11	8-4-34	56.0	175.5	1.69	236.5, 230.0	233.2	67.5	41.7	+ 3.5	+ 1.7	- 1.1
12	9-10-33	48.5	168.6	1.54	209.6	209.6	60.68	39.4	+ 1.5	± 0	± 0
12	21-10-33	48.5	168.6	1.54	173.5, 181.5	177.5	51.35	33.3	- 13.5	- 15.7	- 14.5
13	12-10-33	46.5	168.3	1.51	169.5	169.5	49.05	32.5	- 16.0	- 17.7	- 17.0
14	12-10-33	43.2	166.0	1.45	210.0	210.0	60.8	41.9	+ 8.0	+ 2.3	+ 8.0
15	14-10-33	45.5	168.6	1.49	199.0	199.0	57.6	37.9	- 1.0	- 4.5	- 3.0
16	15-10-33	52.0	166.6	1.57	209.5, 211.0	210.2	60.8	38.7	- 1.0	- 2.0	- 1.7
16	31-7-34	53.3	167.1	1.59	234.0, 231.0	232.5	67.35	42.35	+ 8.0	+ 7.2	+ 7.0

16	1-8-34	53.3	167.1	1.59	221.0, 219.0	220.0	63.7	40.0	+	2.0	+	1.5	+	1.5
17	21-10-33	49.0	160.2	1.49	181.0, 175.0	178.0	51.5	34.6	—	11.0	—	12.5	—	11.0
18	18-10-33	56.5	178.6	1.70	207.5, 215.5	211.5	61.25	36.0	—	8.0	—	8.8	—	11.0
19	24-3-34	52.5	170.0	1.60	207.0, 211.5	209.2	60.5	37.8	—	1.0	—	4.3	—	3.5
19	25-3-34	52.5	170.0	1.60	216.0, 203.0	209.5	60.65	37.9	—	1.0	—	4.0	—	3.5
20	24-3-34	71.3	177.5	1.88	232.0, 230.0	231.0	66.9	35.6	—	10.0	—	10.0	—	9.5
20	25-3-34	71.3	177.5	1.88	228.0, 228.0	228.0	66.0	35.1	—	11.1	—	11.0	—	11.0
21	31-7-34	53.0	177.0	1.65	199.0, 199.0	199.0	57.6	34.0	—	9.0	—	11.6	—	11.0
21	1-8-34	53.0	177.0	1.65	196.0, 186.0	191.0	55.3	33.5	—	14.0	—	17.7	—	14.5
22	27-9-34	55.5	174.50	1.67	207.5, 203.0	205.2	59.3	35.5	—	9.0	—	10.0	—	10.0
23	28-8-34	63.0	174.5	1.76	224.5, 217.5	221.0	64.0	36.4	—	8.0	—	7.6	—	8.3
24	24-8-34	57.0	161.0	1.62	184.0, 182.0	183.0	53.0	32.7	—	17.0	—	17.2	—	16.5
25	14-8-33	54.0	175.5	1.66	187.0, 191.0	189.0	51.7	33.0	—	10.0	—	16.4	—	10.2
26	22-8-34	57.2	162.0	1.61	214.0, 211.0	212.5	61.55	38.2	—	2.5	—	3.3	—	2.0
26	23-8-34	57.2	162.2	1.61	208.0, 202.0	205.0	59.3	36.8	—	6.0	—	7.0	—	5.5
27	9-10-34	46.5	164.5	1.50	209.0, 194.0	201.5	58.35	38.9	+	0.6	—	1.5	+	0.5
28	9-10-34	41.5	168.0	1.43	178.5, 168.0	173.2	50.1	35.0	—	11.0	—	14.6	—	7.0
29	12-10-34	56.2	172.0	1.66	197.5, 195.0	196.2	56.7	34.2	—	12.0	—	13.4	—	11.5
30	12-10-34	60.2	169.5	1.63	196.5	196.5	56.8	34.8	—	9.2	—	11.9	—	10.0
31	27-10-34	58.2	162.5	1.73	217.0, 218.5	217.7	63.0	36.4	—	6.5	—	7.8	—	6.5
32	27-10-34	68.2	167.0	1.76	228.5	228.5	66.15	37.5	—	6.0	—	4.9	—	4.0

irregular. Often tests on a subject were made on two or more different days. This was deemed especially necessary when high readings were obtained, to make sure that the high results were not due to any error in technique.

The average oxygen consumption for all the 32 subjects works out as 200.4 c.c. per minute.

Heat production per hour.—Column 8 of Table IV gives the total heat production of the body per hour from which the heat production per square metre of the body surface was calculated. This is given in column 9. The heat production was computed from the oxygen consumption. It was assumed that the average respiratory quotient was 0.82 in which case it has been calculated that each litre of oxygen gives rise to the production of 4.825 calories. For the purpose of expressing the amount of oxygen in terms of calories per hour, use was made of the excellent table contained in the Sanborn Company's handbook (Sanborn, 1933). The average heat production per square metre of the body surface per hour for all the 32 subjects works out as 36.16 calories.

Basal metabolism.—The basal metabolism is expressed here as the percentage deviation from two commonly accepted standards, viz., Aub-DuBois and Harris-Benedict. Comparison with the so-called Sanborn normals has also been made.

In order to compare the results obtained in the case of my subjects with the Harris-Benedict standards, I have made use of the tables published by the Carnegie Institution of Washington and abridged and re-arranged by Sanborn (*loc. cit.*).

The results are shown in Table IV. Here each day's result is given separately. In Table II is given the average result for each subject of the different days that the tests were taken. From this it is seen that only five of the 32 subjects give positive results. All the rest give negative results. The lowest results obtained are in the subjects Nos. 1, 13 and 24. The average for all the 32 subjects works out as 6.8 per cent lower than the Harris-Benedict standards and 8.7 per cent lower than the Aub-DuBois standards. The Aub-DuBois standards are, therefore, only 1.9 per cent higher than the Harris-Benedict standards so far as their application to these subjects is concerned.

According to Krogh's tables (1920), a uniform reduction of 6 per cent is made from Aub-DuBois standards for the ages of 15 to 75 years. It has been brought out by recent investigations that, for ages of 20 years and thereabout, the Aub-DuBois values are nearly the same as those of Harris-Benedict. The results obtained in my subjects tend to support this statement.

In the case of subjects Nos. 19, 20, 21 and 26, tests were made on two consecutive days. It is seen that, except in the case of subject No. 19, the second day's results are lower than the first day's results. This I do not believe to be due to a greater mental or muscular repose obtained on the second day, as these subjects were apparently quite at ease on the first day also. The lower results obtained on the second day are probably due to a feeling of fatigue as the result of experiments on two successive days. In the subjects Nos. 1, 2, 3, 6, 11, 12 and 16, tests were made on two or more widely different days. It is seen that the results of the second or subsequent tests are not necessarily lower than the first tests and also that the difference in the results is, sometimes, considerable. This might be due to the psychical state of the subject as has been recently brought out by Benedict (1935).

His own metabolism was measured on eighteen consecutive days in May 1932 and on thirty-three consecutive days in May and June 1933. The results were extraordinarily uniform from day to day. Only on five out of these fifty-one days there were marked variations which were attributed by the author to have been caused by emotional disturbances.

COMPARISON WITH THE BASAL METABOLISM RESULTS OBTAINED IN INDIA BY OTHER INVESTIGATORS.

It has already been stated that Mukherjee (*loc. cit.*) found, in 15 Bengalis, the basal metabolism 9 per cent lower than the Sanborn normals. And again, Mukherjee and Gupta (*loc. cit.*), studying 18 normal Bengali young men, obtained results 13·3 per cent below Aub-DuBois standards. Sokhey (*loc. cit.*) in Bombay obtained results 10 to 23 per cent below the Aub-DuBois standards, but as the details are not given, it is not possible to compare the present results with those obtained by Sokhey. Mason's and Benedict's (1931) results are the lowest of all, their 54 women subjects giving an average value of 16·9 per cent below Harris-Benedict standards and 17·2 per cent below those of Aub-DuBois. Banerji (1931) studied 145 prisoners of the district jail, Lucknow, and found the average basal metabolism to be 6·9 per cent below the 'European standards'. Banerji finds that during the monsoon season, when the atmosphere is very humid, the basal metabolism values are lower than those obtained during the drier seasons. He, however, does not mention which standards he had actually compared his results with. In any case it would be doubtful if the results obtained from prisoners in an Indian prison can be compared with the results obtained usually from students.

It is thus seen that the results obtained in my subjects are higher than those obtained in Bombay, Madras and Calcutta. They are about 5 per cent higher than the results obtained by Mukherjee and Gupta at Calcutta and about 9 per cent higher than the results obtained by Mason in the South Indian women. It may here be pointed out, however, that, so far, enough work has not been done in India to establish the sex difference in metabolism. It is quite possible, as DuBois (*loc. cit.*) suggests, that the sex difference is greater in the Oriental. This view is supported by the studies of Takahira who found the basal metabolism of the Japanese women 9·3 per cent less than that of the Japanese men : whereas the sex difference for the Americans has been worked out as about 7 per cent (Gephart and DuBois, 1916 ; Harris and Benedict, *loc. cit.*). Krishnan and Vareed (1932) studied 76 medical students in Madras of whom 15 were women. Their ages varied between 18 and 25 years. They found that the average basal metabolism of their male subjects was 12 per cent and of their female subjects 16 per cent below the DuBois standards. This gives a sex difference of 11 per cent.

It is thus seen that the results obtained by me at Hyderabad are higher than those obtained elsewhere in India with the exception of Lucknow. Nevertheless, these results are lower than the standards accepted for Westerners. The cause of this low metabolism in the Indians has been attributed to various factors, viz., climatic, dietetic, occupational and racial.

Climatic factor.—Is climate a factor in determining the basal metabolism ? This has already been discussed to some extent, but the evidence on this point is

conflicting. There is evidence, however, to believe that a prolonged residence in a warm climate tends to lower the basal metabolism. Earle (1928) reported the results of the studies made by him and his colleagues on 166 Chinese and also on 41 Westerners in Hong Kong and Peking. The basal metabolism of the Chinese subjects was found to be 8 per cent below the DuBois standards and that of Westerners in Hong Kong and Peking 7 per cent below the DuBois standards. On the other hand 5 Orientals examined by Earle in London were found to have basal metabolism 6.5 to 8.5 per cent below DuBois, Harris-Benedict and Dreyer standards. These results therefore 'appear to afford evidence of a climatic factor as the controlling influence in basal metabolism'.

In a recent publication Mason (*loc. cit.*) reports that the average decrease in metabolism in a group of nine European women moving to the tropics was 5.1 per cent and that three Indian women measured in two climates showed an increase in metabolism of 4.8 per cent in cold climates.

The influence of warm moist climate in lowering metabolism is suggested by Banerji (*loc. cit.*). If this is accepted, then it would also explain why the basal metabolism results are higher at Hyderabad than at the damp seaside places like Bombay, Calcutta and Madras. Table V gives the basal metabolism results with reference to the humidity of the air. It is seen that the average basal metabolism for those days in which the difference between the wet- and the dry-bulb readings was 4°C. or more works out as 4.4 per cent below the Harris-Benedict standards; whereas the average for those days in which this difference is less than 4°C. is 7.1 per cent below the Harris-Benedict standards. These experiments, however, are not of much value being few in number and not undertaken especially to show the effect of humidity on the basal metabolism. They, nevertheless, point to the possibility of humidity being a factor in modifying the basal metabolism. Further work is needed to elucidate this point.

TABLE V.

Subject num- ber.	Date.	Dry-bulb temperature, °C.	Wet-bulb temperature, °C.	DEVIATION FROM HARRIS- BENEDICT.	
				Per day.	Average.
2	8-4-34	31.5	24.5	- 4.5	- 4.5
11	8-4-34	32.0	24.5	+ 3.5	+ 3.5
19	24-3-34	28.5	20.0	- 1.0	} - 1.0
19	25-3-34	28.5	22.0	- 1.0	
20	24-3-34	29.5	21.0	- 10.0	} - 10.5
20	25-3-34	28.5	22.0	- 11.0	
27	9-10-34	29.0	25.0	+ 0.6	+ 0.6
28	9-10-34	29.0	25.0	- 11.0	- 11.0
31	27-10-34	25.0	19.5	- 6.5	- 6.5
32	27-10-34	26.5	21.0	- 6.0	- 6.0
AVERAGE					- 4.4

TABLE V--concl'd.

Subject num- ber.	Date.	Dry-bulb temperature, °C.	Wet-bulb temperature, °C.	DEVIATION FROM HARRIS- BENEDICT.	
				Per day.	Average.
1	8-9-33	..	..	- 9.0	} - 14.5
1	14-10-33	28.0	25.5	- 20.0	
2	8-9-33	..	..	- 9.0	- 9.0
3	8-9-33	..	..	+ 7.0	} + 2.7
3	18-10-33	27.0	25.5	- 1.0	
3	22-8-34	25.0	22.5	+ 2.0	- 5.0
4	27-9-33	..	..	- 5.0	- 1.0
5	27-9-33	..	..	- 1.0	} + 2.7
6	27-9-33	..	..	+ 10.0	
6	8-10-33	29.5	26.5	- 4.0	- 17.4
6	18-10-33	27.0	25.5	+ 2.0	- 10.0
7	28-9-33	..	..	- 17.4	- 9.0
8	28-9-33	..	..	- 10.0	- 14.0
9	9-9-33	26.5	25.0	- 9.0	} - 3.0
10	17-8-33	..	..	- 14.0	
11	8-10-33	28.5	26.5	+ 2.0	} - 6.0
11	19-10-33	26.5	25.0	- 8.0	
12	9-10-33	29.0	26.0	+ 1.5	- 16.0
12	21-10-33	24.5	22.0	- 13.5	+ 8.0
13	12-10-33	27.5	24.5	- 16.0	- 1.0
14	12-10-33	28.0	24.5	+ 8.0	} + 3.0
15	14-10-33	28.0	25.5	- 1.0	
16	15-10-33	28.0	25.5	- 1.0	- 11.0
16	31-7-34	25.0	24.0	+ 8.0	- 8.0
16	1-8-34	25.0	24.0	+ 2.0	} - 3.5
17	21-10-33	25.0	22.5	- 11.0	
18	18-10-33	27.0	25.5	- 8.0	- 14.0
21	31-7-34	25.5	24.5	- 9.0	- 17.0
21	1-8-34	25.0	24.0	+ 2.0	- 10.0
23	28-8-34	27.0	24.0	- 14.0	} - 4.2
24	28-8-34	27.2	24.2	- 17.0	
25	14-8-34	..	..	- 10.0	- 12.0
26	22-8-34	26.5	24.0	- 2.5	- 9.2
26	23-8-34	25.5	24.0	- 6.0	
29	12-10-34	28.0	25.0	- 12.0	
30	12-10-34	28.0	25.0	- 9.2	
AVERAGE					- 7.1

Dietetic factor.—Evidence has accumulated that protein-rich diet is a potent factor in raising the basal metabolism. This was brought forward by Krogh and Lindhard (1920). Heinbecker (1928) found the metabolism of Eskimos, who are heavy protein consumers, to average 33 per cent above the normal standards. Benedict and Roth (1915), however, examined 11 male and 11 female vegetarians and found that the metabolism did not differ significantly from that of the non-vegetarians. By vegetarians was meant persons who did not eat meat of any kind more than two or three times a year. These results were confirmed by Harris and Benedict (*loc.-cit.*).

Out of my 32 subjects, 8 are vegetarians. Out of these 8, Nos. 20 and 25 abstain totally from meat but take eggs occasionally. The rest of the 8, from Nos. 27 to 32 inclusive, abstain totally both from meat as well as eggs. These subjects belong to castes which have been vegetarians for generations. The average basal metabolism for all these 8 subjects works out as 8.08 per cent below the Harris-Benedict standards and 10.06 per cent below the Aub-DuBois standards. Vegetarian habits as such, therefore, seem to have a somewhat depressing effect on basal metabolism.

Occupational factor.—It is possible that the low metabolism rates obtained in India may, in part, be due to a less active life of the Indian subjects. Most of the tests made in India are on college students and it cannot be said with certainty that the college students in India lead a less active life than students in colder climates. It is only surmised that the warmer climate of India would lead to a less active life. That athletic training raises the rate of the basal metabolism has been observed by many workers (Benedict and Smith, 1915; Gephart and DuBois, *loc. cit.*; Lauter, 1926; etc.) Against this, Tilt (1930) found the basal metabolism of 10 athletic women in Florida to be no higher than that of the whole mixed group. And Krishnan and Vareed (*loc. cit.*) found that in the case of 6 of their subjects, 'who were daily indulging in fairly severe exercise', the basal metabolism was distinctly lower than that of the mixed group.

Racial factor.—In 1925, MacLeod, Crofts and Benedict (*loc. cit.*) emphasized for the first time the existence of a racial factor. They examined 7 Chinese and 2 Japanese women students in America and found their basal metabolism to be 10.4 per cent below the Harris-Benedict standards. As these standards have been considered to be 5 per cent too high for American college women, this leaves the Oriental women students only 5 per cent below their American colleagues. This difference was considered to be due to the existence of a racial factor. Subsequently the Nutrition Laboratory of the Carnegie Institution of Washington has embarked on a comprehensive programme for the study of the basal metabolism of various races. From the results obtained, Benedict and his co-workers of the Nutrition Laboratory have come to a definite conclusion that a racial factor exists. There are, however, difficulties in the unqualified acceptance of this conclusion.

An important series of investigations has been carried out on the Mayas of Yucatan. On three different expeditions it was found that the basal metabolism in the Mayas was definitely higher than in the case of the North American whites. And, what is more, there was a definite suggestion that a Maya of purer extraction tended to have a higher metabolism than one less pure. This, however, has not been definitely settled. Recently, Pi-Suner (1933) has obtained similar results on another aboriginal tribe, the Mapuche Indians of southern Chile. In 31 of his male subjects he found an average basal metabolism 9.8 per cent higher than the Harris-Benedict standards. The Nutrition Laboratory workers have contracted the high basal metabolism of the Maya males with the very low basal metabolism of the South Indian females and conclude that such a wide difference cannot but be due to a racial factor.

There is, however, reason to believe that in the case of both the Mayas and the Mapuches there are other factors which would tend to raise the basal metabolism. Both the Mayas and the Mapuches are aboriginal tribes. The living conditions

amongst them are very poor. 'The Maya sleeps inadequately protected by his meagre clothing and his hammock against the cold nights'. The same may be said of the Mapuches of southern Chile where the temperature goes down in September (when the above-mentioned observations were made) to the freezing point. It is quite possible that in the case of these aboriginal tribes the higher basal metabolism is a result of a compensatory mechanism to offset the effect of exposure to cold nights.

Recently Benedict and Meyer (*loc. cit.*) have examined 18 American-born Chinese girls (12 to 22 years of age) living in the United States. They find that these girls have a basal metabolism averaging 9.2 per cent lower than that of the American girl scouts of the same ages and 6.1 per cent lower than the Aub-DuBois standards for American girls. Now if it is conceded, as has been suggested, that the Aub-DuBois standards are 5 per cent too high even for American women then these Chinese girls have metabolism averaging practically the same as Aub-DuBois predictions. On the other hand the metabolism of the girl scouts was taken while they were asleep. It is therefore doubtful whether it is quite fair to apply the girl scouts' standards (with 10 per cent correction for sleep) in the case of the Chinese. Besides this, the authors admit that these American-born Chinese girls were not free from Chinese dietetic habits. Under the circumstances it cannot be said that these tests definitely establish the existence of a racial factor. In the words of the authors themselves, 'until further measurements are made upon other girls (both Chinese and Caucasians) in this age range, however, the drawing of any final conclusion must be held in abeyance'.

If we now review the numerous observations that have been made on different races in different countries we can have some idea of how far the racial factor operates. The following are some of the main observations:—

Observations.	DEVIATION FROM NORMAL STANDARDS.	
	Harris-Benedict.	Aub-DuBois.
1. Eijkmann's observations on the Malays in Batavia.	..	— 0 per cent.
2. Takahira's observations on the Japanese men.	..	— 5.5 „
3. Earle's observations on the Chinese.	..	— 8.0 „
4. Mason's observations on the Madrassce women.	— 16.9	— 17.2 „
5. Mukherjee and Gupta's observations on the Bengali young men.	..	— 13.3 „
6. Observations on the Hyderabadi young men.	— 6.8	— 8.7 „

These figures may be compared with the following figures obtained on the Europeans in the East :—

Observations.	DEVIATION FROM NORMAL STANDARDS.	
	Harris-Benedict.	Aub-DuBois.
1. Montoro's observations on the white Cubans in Havana (quoted by DuBois, 1927).	— 15·8	— 15·5 per cent.
2. de Almeida's observations on white men in Brazil.	— 13·6	— 16·2 „
3. Mason's (1934) observations on the European women in Madras.	— 7·9	— 12·5 „
4. Earle's report on European men at Hong Kong and Peking.	..	— 7·0 „

The comparison of the two sets of figures shows how closely they resemble each other. Had the racial factor been an important factor in determining the basal metabolism, it would seem strange that the basal metabolism figures of races so widely different as the Caucasians of Europe and America and the Mongolians of China and Japan should tally so closely. It would be more reasonable to suppose that though a racial factor may exist it is probably not so important as the climatic and the environmental factors in determining basal metabolism.

CAUSE OF THE LOW BASAL METABOLISM IN THE INDIANS.

Very few observations have so far been made in India and these go to show the existence of a lower basal metabolism in the Indians. Probably all the causes discussed above operate in bringing about this low level. Mukherjee and Gupta (*loc. cit.*) have attributed it to climatic and nutritional causes. McCarrison (1927) studied the dietary habits of the different Indian provinces and found that the Bengali, the Kanaree and the Madrased diet is of low biological value and is poor in the contents of suitable proteins, vitamins and mineral elements. The lowest figures have been obtained in the Madrased women. But if a 5 per cent correction is made to these figures on the ground that the Aub-DuBois and the Nutrition Laboratory predictions are 5 per cent too high for women, then the average works out as 11 to 13 per cent which is about the same as for the Madrased men.

Mason and Benedict (1931) attributed the low metabolism of the Madrased women to the existence, at least in part, of a definite racial factor. Other possible factors which they suggest are : a low protein diet, tropical conditions of climate and a state of relaxation during repose as complete as that which is found during sleep among Westerners.

In a later publication Mason and Benedict (1934) give the results of their investigations of the basal metabolism in 7 Madrassese women while awake and during sleep, and have compared this result with that found in 2 Western women living in Madras under the same conditions. They found that in the Madrassese women the oxygen consumption during sleep decreased on an average 9·8 per cent. The two Western women gave similar results. The authors conclude that the state of relaxation is not the causal factor in the low basal metabolism of the Madrassese women. This conclusion seems to be justified, however, only if it can be proved that the amount of muscular relaxation during sleep is the same in both the Oriental and the Occidental.

SUMMARY AND CONCLUSIONS.

Basal metabolism tests and physical character studies have been carried out in 32 young men at Hyderabad (Deccan). The ages varied from 19 to 31 years, the average being 22 years.

No difference was found in the pulse and the respiration rates as compared with the Western standards.

The subjects were found to have somewhat (about 1 cm.) shorter stem height and correspondingly longer legs compared to the measurements obtained in the Westerners.

A pelidisi of about 92 indicates the normal state of nutrition in young Indians, the average pelidisi for 30 out of the 32 subjects being 92 ± 4 .

The blood pressure was found to be considerably lower compared to the Western standards. The average systolic blood pressure in 30 out of the 32 subjects was $100\cdot4 \pm 11$ mm., the average diastolic pressure $71\cdot8 \pm 5$ mm., and the average pulse pressure $28\cdot6 \pm 7\cdot2$.

The basal metabolism was found to be 6·8 per cent below the Harris-Benedict and 8·7 per cent below the Aub-DuBois standards. The factors which may tend to bring about this low level of metabolism are discussed.

The possibility of the humid climate being a factor in lowering metabolism is suggested.

Eight of the subjects who were vegetarians showed the basal metabolism on an average about 2 per cent lower than that obtained for the whole mixed group.

For height and age, the weight in this group of subjects was less than the American standards.

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FACTORS AFFECTING THE CAROTENE CONTENT OF CERTAIN VEGETABLE FOOD-STUFFS.

BY

N. K. DE.

(From the Nutrition Research Laboratories, I. R. F. A., Coonoor.)

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IN a previous communication (De, 1936), it was pointed out that non-leafy vegetable foods, such as roots, pulses and fruits, do not lose carotene (provitamin A) appreciably during a reasonable period of storage. In certain foods, indeed, development of carotene may continue until long after harvesting; ultimately, however, deterioration takes place. The green leafy vegetables, which are very rich in carotene, were found to lose a high proportion of their carotene even during a few days' storage under ordinary conditions. The recognition of the importance of vitamin A in the feeding of live stock and poultry has resulted in a considerable amount of work on the variation of the vitamin A values of alfalfa hays and meal (Hauge and Aitkenhead, 1931; Russell, 1929; Guilbert, 1935), but little similar work has been done on human foods. Guilbert (*loc. cit.*), in his extensive work on alfalfa hays and meal, using a colorimetric method of assaying carotene, investigated the influence of various factors, viz., light (visible and ultra-violet), temperature, bacterial and enzyme action, on the carotene content of samples during storage. Little or no loss of carotene occurred during vacuum-drying even at a temperature of 100°C. Guilbert's figures show that the rate of destruction of carotene in dried alfalfa during storage was negligible as compared to that occurring in fresh samples.

Both vitamin A and carotene are known to be stable to heat of ordinary degree in the absence of oxygen. Yellow corn, carrot, sweet potato and squash have been heated for 3 hours at 250°F. with no loss of vitamin A potency. The potency of most canned foods was found to be practically undiminished (Sure, 1933). According to Steenbock and Boutwell (1920), Coward and Morgan (1935) and many others, vegetable foods do not lose appreciable amounts of vitamin A in the domestic cooking or canning processes.

Germination of grains like corn, barley and mung has not been found to produce any noticeable variation in their vitamin A potency; an increased potency was, however, noted when the germinated seeds formed leaves (Karshan, Krasnow and Harrow, 1927; Coward and Drummond, 1921; Miller and Hair, 1928).

In most of the work quoted above, the vitamin A value of test materials was estimated by feeding experiments. It is now established that the vitamin A potency of vegetable food-stuffs is mainly due to carotene (precursor of vitamin A), and there have already been introduced several laboratory methods, chemical or physical, for assaying carotene which probably give more accurate results than any that can be obtained by biological tests.

In the present work the variation of carotene in a few edible leafy vegetables during storage under various conditions was studied, using the spectrophotometric method previously described (De, 1935, 1936). Simultaneously the influence of other factors, viz., milling, parboiling, cooking and sprouting, was also investigated.

EXPERIMENTAL.

The various factors, the influence of which on carotene content was studied, are described in Tables I to V. The leafy vegetables were obtained fresh from a neighbouring garden and immediately used for experiment; the other test materials were bought in the local market. As far as possible, identical specimens of the same material were used throughout each series of experiments. Results are expressed in γ (0.001 mg.) per gramme of initial weight.

TABLE I.

Effect of various factors (parboiling, husking, light, temperature, bacterial action, etc.) on the carotene content of certain vegetable food-stuffs.

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).	Percentage loss of carotene.
1	Unmilled raw rice, husk removed from paddy by hand	0.34	..
	Unmilled parboiled rice, husk removed from paddy by hand.	0.26	23.5
	Raw rice, from same paddy, home-pounded ..	0.04	90.0
	Parboiled rice, from same paddy, home-pounded ..	0.15	55.5
	Highly milled rice (different sample)	Nil	100.0
2	Amaranth (<i>Amaranthus gangeticus</i>), fresh leaves ..	68.16	..
	Leaves preserved in dark in a vacuumized desiccator for 2 days at 0°C.	65.10	4.5
	do. do. for 2 days at 15°C. to 22°C.	64.30	5.7
	Leaves preserved in paper packets for 2 days at 15°C. to 22°C. (room temperature).	61.60	9.6
	do. do. 3 days at 15°C. to 22°C.	54.12	20.6
	Leaves exposed to diffused sunlight in the laboratory for 2 days.	51.70	24.1
	do. do. for 3 days ..	44.00	35.4

TABLE I—*contd.*

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).	Percentage loss of carotene.
	Leaves kept under water containing a little formaldehyde for 3 days at 15°C. to 22°C. (remained green and fresh).	63.34	7.1
	Leaves kept under water for 3 days at 15°C. to 22°C. (remained green and fresh).	49.60	27.2
	Leaves kept under water containing toluene for 24 hours at 15°C. to 22°C. (greenness and freshness lost).	40.60	40.4
3	Amaranth, fresh leaves	61.60	..
	Leaves preserved under water containing a little formaldehyde for 24 hours at 38°C. (remained green and fresh).	59.70	3.1
	do. with toluene for 24 hours at 38°C. (greenness and freshness lost).	17.60	71.3
	Leaves kept under water containing formaldehyde for 3 days at 38°C.	56.60	8.1
	Leaves kept under water alone for 3 days at 38°C.	28.00	53.6
	Leaves kept in paper packets for 3 days at 38°C. ..	35.55	42.5
4	Celery leaves, fresh	74.70	..
	Leaves stored in a vacuumized desiccator at room temperature for 24 hours.	73.14	2.1
	do. do. for 2 days	70.40	5.8
	Leaves preserved in paper packets in the laboratory at room temperature for 5 days.	45.70	32.8
	Leaves preserved under water containing formaldehyde for 5 days at room temperature.	66.40	11.1
5	Coriander (<i>Coriandrum sativum</i>), fresh leaves ..	116.70	..
	Leaves preserved under water containing a little toluene at room temperature for 24 hours.	64.80	44.6
	Leaves preserved in an atmosphere of CO ₂ gas under water containing a little toluene at room temperature for 2 days.	105.00	10.0
	Leaves preserved under water containing a little formaldehyde at room temperature for 4 days.	99.00	15.2
	Leaves preserved in paper packets at room temperature for 4 days (became dried up and unfit for use).	70.00	40.0
	Leaves sprinkled with water and preserved at room temperature wrapped in green plantain leaf for 2 days (remained quite green and fresh).	112.00	4.0
	do. do. for 4 days (part became rotten; rest remained green and fresh).	70.00	40.0
	Leaves sprinkled with water and preserved at room temperature (wrapped in wet cloth) for 2 days.	110.00	5.7
	do. do. for 4 days (part became rotten; rest remained green and fresh).	72.00	38.3

TABLE II.

Effect of storage at various temperatures on the carotene content of coriander leaves (Coriandrum sativum).

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).	Percentage loss of carotene.
1	Leaves, fresh	103.6	..
	Leaves, vacuum-dried at 100°C.	104.0	..
2	Leaves preserved in paper packets in the laboratory for 5 hours at 0°C.	102.0	1.5
	do. at 15°C. to 22°C. (room temperature) ..	100.2	3.3
	do. at 38°C.	94.3	8.9
3	Leaves preserved in paper packets in the laboratory for 24 hours (1 day) at 0°C.	99.0	4.4
	do. at 15°C. to 22°C.	88.7	14.4
	do. at 38°C.	82.0	20.8
4	Leaves preserved in paper packets in the laboratory for 2 days at 0°C.	90.0	13.1
	do. at 15°C. to 22°C.	75.0	27.6
	do. at 38°C.	65.0	37.3
5	Leaves preserved in paper packets in the laboratory for 3 days at 0°C.	79.5	23.3
	do. at 15°C. to 22°C.	68.6	33.8
	do. at 38°C.	57.6	44.4
6	Leaves preserved in paper packets in the laboratory for 5 days at 0°C.	68.7	33.7
	do. at 15°C. to 22°C.	57.7	44.3
	do. at 38°C.	45.4	56.2
7	Leaves preserved in paper packets in the laboratory for 12 days at 0°C.	61.5	49.6
	do. at 15°C. to 22°C.	49.8	51.9
	do. at 38°C.	40.5	60.9

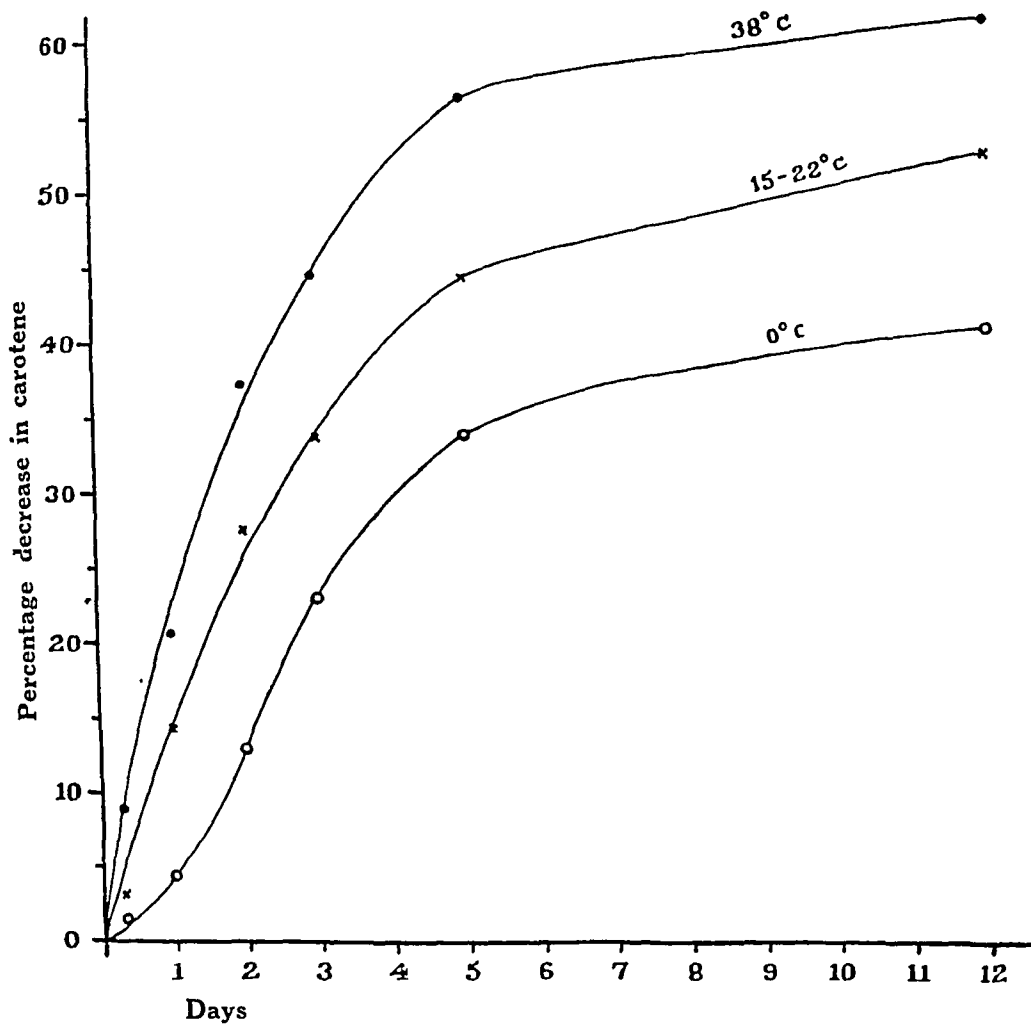


FIG. 1. Percentage decrease in the carotene content of coriander leaves stored in paper packets in the laboratory at 0°C., 15°C. to 22°C., and 38°C., respectively.

TABLE III.

Effect of storage at various temperatures on the carotene content of spinach leaves.

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).	Percentage loss of carotene.
1	Fresh leaves	34.84	..
	Leaves, vacuum-dried at 100°C.	34.71	..
2	Leaves preserved in paper packets in the laboratory for 5 hours at 0°C.	33.50	3.8
	do. at 15°C. to 22°C. (room temperature) ..	32.70	6.1
	do. at 38°C.	29.88	14.2
3	Leaves preserved in paper packets in the laboratory for 24 hours at 0°C.	32.30	7.3
	do. at 15°C. to 22°C.	28.35	18.6
	do. at 38°C.	22.70	34.8
4	Leaves preserved in paper packets in the laboratory for 2 days at 0°C.	29.20	16.2
	do. at 15°C. to 22°C.	24.00	31.1
	do. at 38°C.	20.10	42.3
5	Leaves preserved in paper packets in the laboratory for 3 days at 0°C.	27.40	21.4
	do. at 15°C. to 22°C.	22.40	35.7
	do. at 38°C.	18.30	47.5
6	Leaves preserved in paper packets in the laboratory for 5 days at 0°C.	24.00	31.1
	do. at 15°C. to 22°C.	18.70	46.3
	do. at 38°C.	16.20	53.5

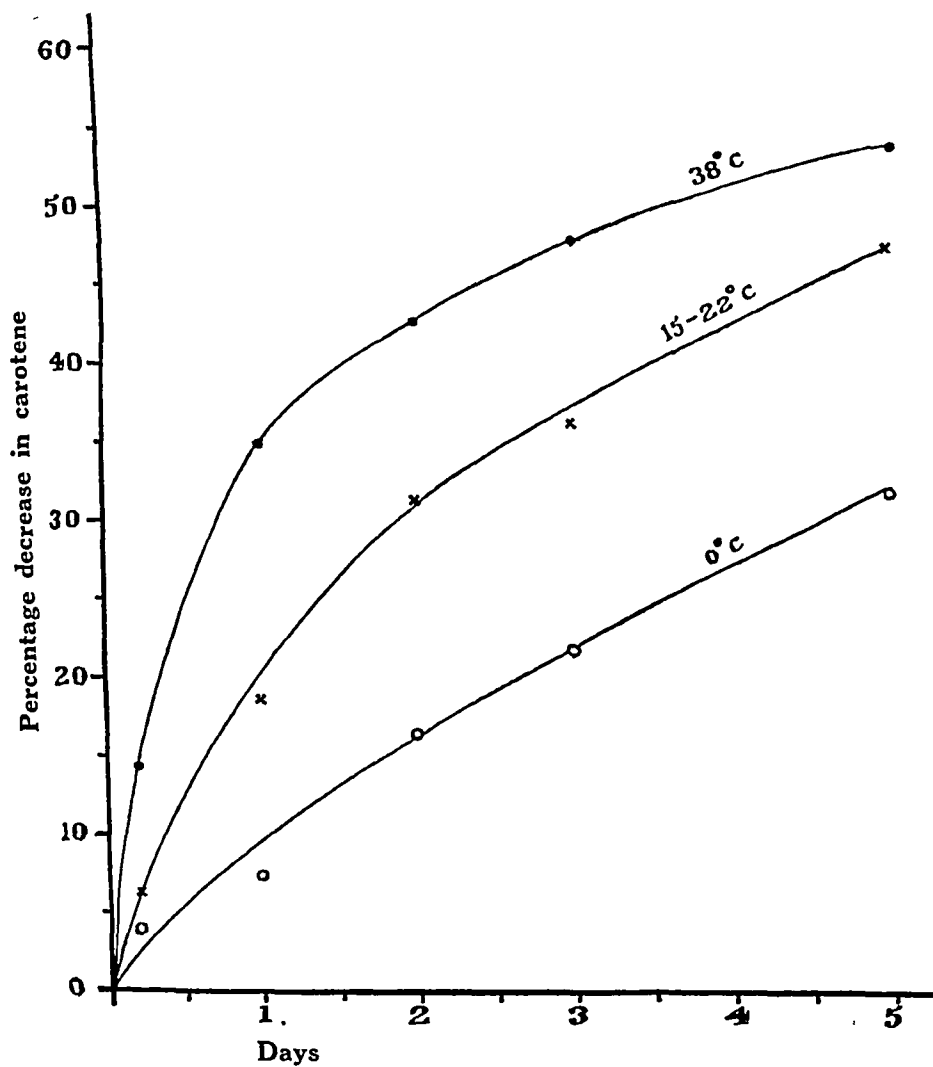


FIG. 2. Percentage decrease in the carotene content of spinach leaves stored in paper packets at 0°C., 15°C. to 22°C., and 38°C., respectively.

TABLE IV.

Effect of cooking on the carotene content of certain vegetable foods.

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).
1	Amaranth leaves (<i>Amaranthus gangeticus</i>) ..	71.70
2	do. do. boiled in tap-water for 30 minutes ..	72.90
3	do. do. for 1 hour ..	70.40
4	Spinach leaves	38.80
5	do. boiled for 1 hour	38.50
6	Potato	0.28
7	do. boiled for 1 hour	0.26
8	Lentils (<i>Lens esculenta</i>)	0.53
9	do. boiled for 1 hour	0.31
10	Red gram (<i>Cajanus indicus</i>)	2.11
11	do. boiled for 1 hour	1.43
12	Unpolished parboiled rice	0.26
13	do. do. boiled for 45 minutes ..	Nil or trace.
14	Ragi (<i>Eleusine coracana</i>), powdered ..	0.61
15	Ragi, powdered, boiled for 45 minutes ..	0.26

TABLE V.

Effect of sprouting on the carotene content of certain pulses.

Test No.	Treatment.	Carotene content per gramme of initial weight in γ (0.001 mg.).
1	Bengal gram (<i>Cicer arietinum</i>)	5.90
2	do. sprouted for 2 days in tap-water (no shoots visible).	5.50
3	do. sprouted for 4 days in tap-water (shoots were visible).	4.80
	do. for 5 days	4.03
	do. " 6 "	3.75
	do. " 7 "	3.40
	do. " 10 "	2.80
	do. " 15 "	2.00
2	do. sprouted for 4 days in 50 mg. per cent $MnCl_2$ solution (shoots were visible).	3.70
	do. do. for 5 days	3.30
3	do. sprouted for 4 days in 50 mg. per cent $MgCl_2$ solution (shoots were visible).	2.10
4	Green gram (<i>Phaseolus radiatus</i>), whole grains ..	4.70
	do. sprouted in tap-water for 1 day ..	3.52
	do. do. 2 days ..	2.15
	do. do. 3 " ..	1.95
	do. do. 5 " ..	1.45
	do. do. 7 days (growth of leaf first visible).	3.52
	do. do. 9 days (leaf visible) ..	8.80

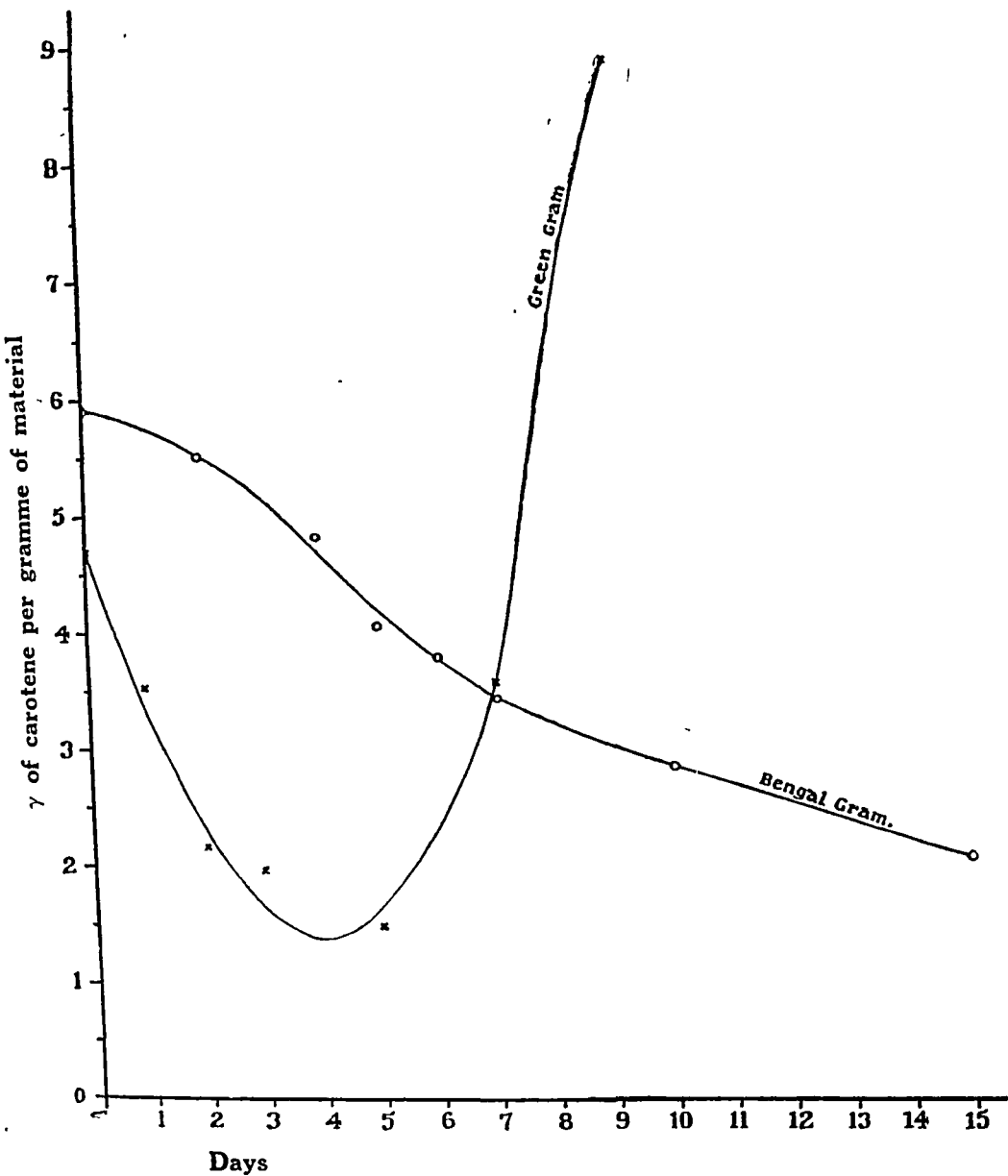


FIG. 3. Carotene content of Bengal gram and green gram sprouted in tap-water for a varying number of days.

DISCUSSION AND SUMMARY.

The process of parboiling causes a slight loss of carotene, which becomes evident when similar samples of unmilled raw and parboiled rice, husked by hand, are compared. 'Home-pounding', which removes most of the germ and a certain proportion of the pericarp, results in a loss. It is noticeable that the carotene content of the home-pounded parboiled rice was superior to that of the home-pounded raw rice, in spite of the initial loss of carotene in the former during the process of soaking, steaming and sun-drying. This is due to the fact that it is easier to separate the husk from parboiled paddy in which the husk is split, than from raw paddy; in the case of the latter more prolonged and vigorous pounding is necessary, the grains are broken, and there is a greater loss of germ and pericarp. Highly milled rice was found to be completely devoid of carotene.

No loss occurred in vacuum-drying of coriander and spinach leaves at 100°C., a finding in agreement with the well-known fact that carotene is relatively stable to heat in the absence of air. Comparatively rapid loss of carotene occurred from exposing fresh amaranth leaves to diffused sunlight. Fresh amaranth leaves preserved under water with a little formaldehyde at 38°C. lost 3 per cent of carotene in 24 hours and 8 per cent in 3 days. Slightly less destruction was noted under similar conditions at room temperature. Leaves preserved under water with a little toluene at 38°C. lost 71 per cent in 24 hours; a 40 per cent loss was noted under similar conditions at room temperature. Guilbert (*loc. cit.*) noted a 95 per cent loss of carotene in incubated alfalfa under water with a little toluene, and ascribed this rapid destruction to strong enzyme action. The results obtained here, however, lead to a different view. The purpose of adding toluene or formaldehyde is to prevent mould or bacterial action. Toluene, being a fat solvent, liberates free carotene from the stored material, and the freed carotene becomes subject to atmospheric oxidation. It was found that destruction of carotene in coriander leaves preserved in water containing toluene was largely prevented in an atmosphere of CO₂ gas. In the case of samples preserved with formaldehyde, no carotene is liberated; consequently only a little destruction takes place. It appears that enzyme action can be only a minor factor in causing destruction of carotene in incubated samples. Amaranth leaves kept under water alone for 3 days lost 27 per cent and 54 per cent of carotene at room temperature (15°C. to 18°C.) and 38°C. respectively, while samples preserved under water with a little formaldehyde lost very little in the same time (7 per cent to 8 per cent). The carotene content of food-stuffs in storage is probably considerably affected by bacterial action.

3 studies on destruction of carotene in coriander and spinach leaves
 4 are at various temperatures are presented in Tables II and III; and
 Figs. 1 and 2. It will be seen that the leafy vegetables lose carotene
 under ordinary conditions at all temperatures, the rate of loss being
 greater at higher temperatures. The modern domestic fridaire thus to some extent
 maintains the vitamin A potency of vegetables, etc. From the shape of the curves
 the rate of destruction gradually decreases as the materials become
 older. Alfalfa leaves can be stored for several weeks with little loss of
 carotene (*loc. cit.*); fresh leaves preserved in paper packets lost
 very little carotene under similar conditions, while samples preserved in *vacuo*

were little affected. These observations lead to the view that mere dehydration in presence of air destroys carotene during storage.

Green vegetables, sprinkled with water and preserved by being wrapped in green plantain leaves or wet cloth, retain a high carotene content for several days. It is interesting to note that this is the usual method employed by the public in India for preserving the freshness of leafy vegetables.

The results of the studies on the effect of cooking are set out in Table IV. The materials were boiled from cold in tap-water for the stated periods. It will be seen that the leafy vegetables and potatoes lost little or no carotene on boiling, while the legumes lost appreciably. The sample of rice was found to be devoid of carotene as a result of cooking for 45 minutes. The destruction of carotene in cooked lentils, red gram, and rice can be accounted for by the fact that the materials on boiling went into solution in the water (in the case of rice the superficial layers of the grain dissolved in the water and the cooked grains became white); the broken tissues were thus easily attacked by heat and atmospheric oxidation.

Table V records data on the effect of sprouting. Several 5-gramme samples of Bengal gram and green gram were kept soaked in tap-water in Petri dishes for 24 hours; afterwards they were spread on separate moist filter-papers which were kept wet by sprinkling water on them occasionally. Green gram showed sprouts after 24 hours, while Bengal gram took over 3 days. The carotene content of the samples was investigated after various periods of sprouting. During the period of observation, Bengal gram sprouts did not form leaves and there was gradual loss of carotene. Similarly green gram showed gradual loss during certain periods of sprouting; when, however, the formation of leaf was visible carotene content showed a sudden rise. The results are set out graphically in Fig. 3.

Dutcher, quoted by Haber and Swanson (1935) in an unpublished investigation, noted that spinach grown in soil containing insufficient amounts of manganese manufactured less vitamin A (carotene) than did spinach grown in the presence of adequate amounts of that element. Magnesium also seems to play some rôle in the formation of plant pigments (Ewart, 1917). Tests Nos. 2 and 3, Table V, show that manganese and magnesium chloride have no marked influence on the carotene content of germinated seeds. The gradual loss of carotene in the pulses during the course of germination may possibly be due to oxidation (bacterial, enzymic or otherwise) or carotene may be used up in the growth of the sprouts of the germinated seeds. The latter inference involves the view that young plants, like young animals, need vitamin A, in the form of carotene, for their growth. Virtanen and his associates (1933), conducting studies on vitamin formation in plants, came to this conclusion. Direct evidence in support of this view is, however, lacking. Whether inorganic salts have any specific effect on the synthesis of carotene, and whether carotene is actually needed for the growth and development of young plants, remains a field for investigation.

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MIGRATION OF ASCORBIC ACID (VITAMIN C) IN AN ELECTRICAL FIELD.

BY

S. RANGANATHAN

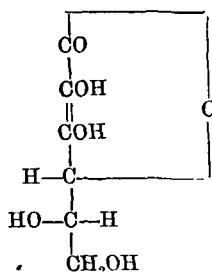
AND

G. SANKARAN.

(From the Nutrition Research Laboratories, I. R. F. A., Coonoor.)

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THE consensus of opinion on the constitution of ascorbic acid, based on both analytical and synthetic studies, is that it has a lactone structure. Its structural formula is :—



It is apparent that no free carboxyl group is present, contrary to the views originally held by certain workers. It is known that lactones are usually broken up in strong alkaline media. In the present investigation the electrical migration properties of ascorbic acid were studied, with the object of discovering whether it behaves as a typical lactone and at what pH this lactone breaks up.

EXPERIMENTAL.

The apparatus used in the present studies is shown in the accompanying Diagram. It consists of a central chamber A, of about 15 c.c. capacity, containing the solution of ascorbic acid in the respective buffers (usually 30 mg. of ascorbic

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acid in about 10 c.c. of the buffer). The central chamber is connected to B and C, the positive and negative electrode vessels, containing the buffer, through side arms provided with taps. The electrode vessels are connected in series to a sodium chloride vessel and a copper sulphate vessel (10 per cent NaCl and CuSO_4 solutions respectively) by means of agar bridges. Two stout copper electrodes dip into the copper sulphate solution. A galvanometer is introduced into the circuit, as shown in the Diagram. The electrodes, being non-polarizable, permit a steady flow of current over long periods. The current is of the order of 3 to 6 milliamperes at 240 volts; in these experiments it was usually passed for about 24 hours. The

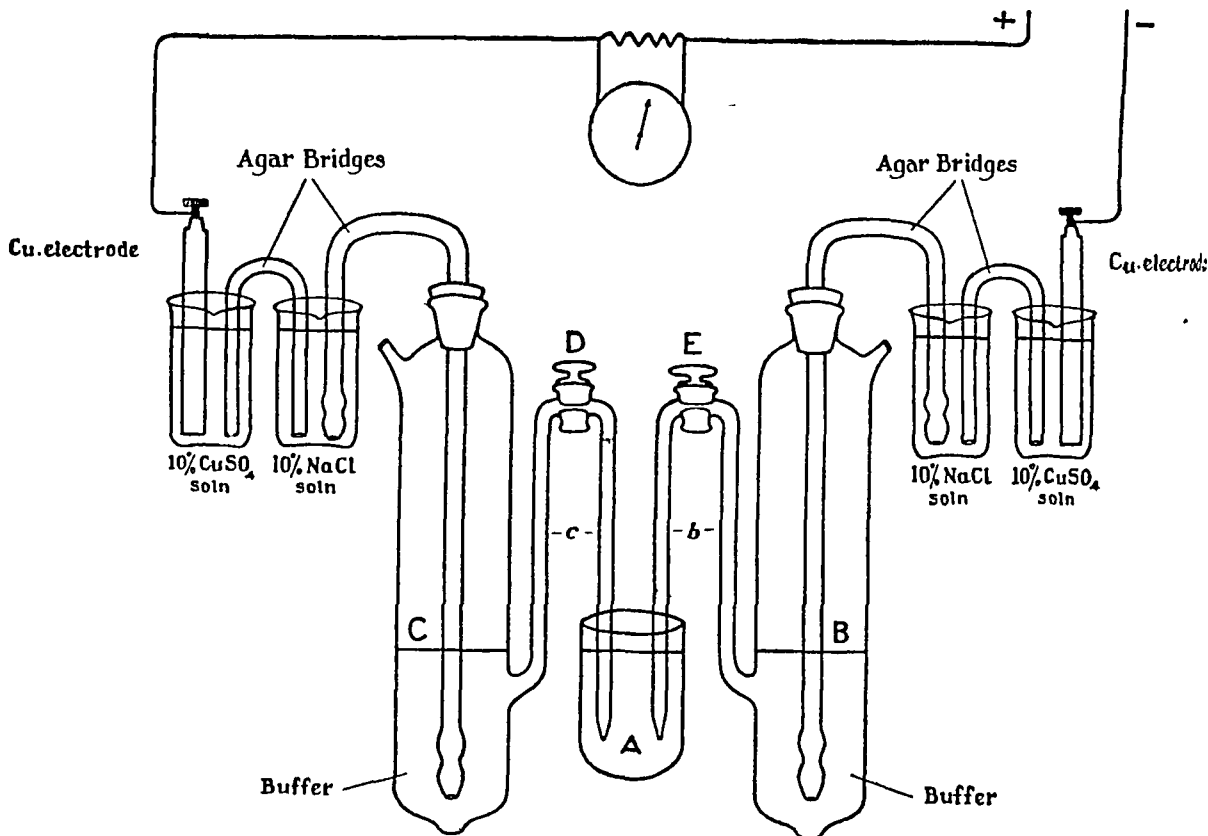


Diagram showing arrangement of apparatus used for electrophoresis.

source of current employed is a 220 volts A. C. street main, converted into 240 D. C. by means of a combined transformer and valve rectifier.

A, B and C are filled with the buffer, care being taken to avoid air bubbles in the side arms of the electrode vessels. The solutions in the three vessels are brought to the same level by opening the taps in the side arms. The taps are then closed and a known amount of buffer is carefully removed from A and replaced by exactly the same volume of ascorbic acid solution in the buffer. The volume of buffer removed from A and that of buffer containing ascorbic acid added must be identical; otherwise siphoning of the ascorbic acid from the central chamber into either electrode vessel may take place.

After running in the current for about 24 hours, taps D and E are closed, and the electrode vessels taken out. A few drops of the liquid from the tips of either electrode vessel are run out into the central chamber, and the outside of the side arms that were dipping into the central chamber washed with distilled water into the central chamber. The contents of A, B and C are then made up to volume, and aliquots titrated against 2:6 dichlorophenol-indophenol, the procedure adopted being that previously used in the laboratory for chemical estimation of vitamin C (Ranganathan, 1935).

The buffers used ranged from pH 1.146 to pH 3.679 and pH 8.575 to pH 12.972, made up of glycoll and either hydrochloric acid or sodium hydroxide, and pH 5.288 to pH 8.043, made up of a mixture of secondary and primary phosphates, according to the tables given in Clark's 'Determination of H-ions' (1920). Varying amounts of current passed through the ascorbic acid solution in the central chamber, depending upon the conductivity of the buffer, being large in the extreme acid and alkaline ranges. The amounts of ascorbic acid migrating are calculated on a uniform basis of 100 milliampere-hours (current strength in milliamperes $\times$ duration of passage of current in hours).

The results of experiments are shown in the accompanying Table and in Figures 1, 2 and 3 :—

TABLE.

pH.		Initial amount in mg. in the central chamber.	Amount in mg. left behind in the central chamber after 24 hours.	Amount in mg. migrated to the +ve chamber per 100 m-a-h.	Amount in mg. migrated to the -ve chamber per 100 m-a-h.	Amount in mg. of ascorbic acid destroyed in 24 hours.	Ascorbic acid migrated, calculated as a percentage of total left at the end of each experiment.
1.146	..	30	22.92	1.54	Nil.	3.78	5.9
2.279	..	30	25.85	1.22	..	2.62	4.5
3.679	..	30	21.29	5.67	..	2.15	20.3
5.288	..	30	15.60	6.17	..	10.00	30.9
5.589	..	30	6.86	10.04	..	11.54	52.9
6.468	..	30	8.02	10.13	..	10.37	51.5
7.381	..	30	12.76	10.50	..	11.33	56.3
8.043	..	30	6.30	8.98	..	14.06	56.2
10.140	..	30	2.11	6.75	..	19.13	62.0
11.067	..	30	5.20	6.70	..	16.39	49.4
12.095	..	30	0.55	3.26	..	24.46	58.9
12.972	..	30	0.00	0.36	..	29.42	62.0

It is apparent from the above Table that ascorbic acid migrated to the positive pole in every instance, behaving consistently as an electro-negative substance. The amounts which migrated varied with the pH and the rate of destruction of ascorbic acid.

Figure 1 shows that there is a progressive increase in the amount of ascorbic acid migrating to the positive chamber up to a pH of about 7, followed by a steady

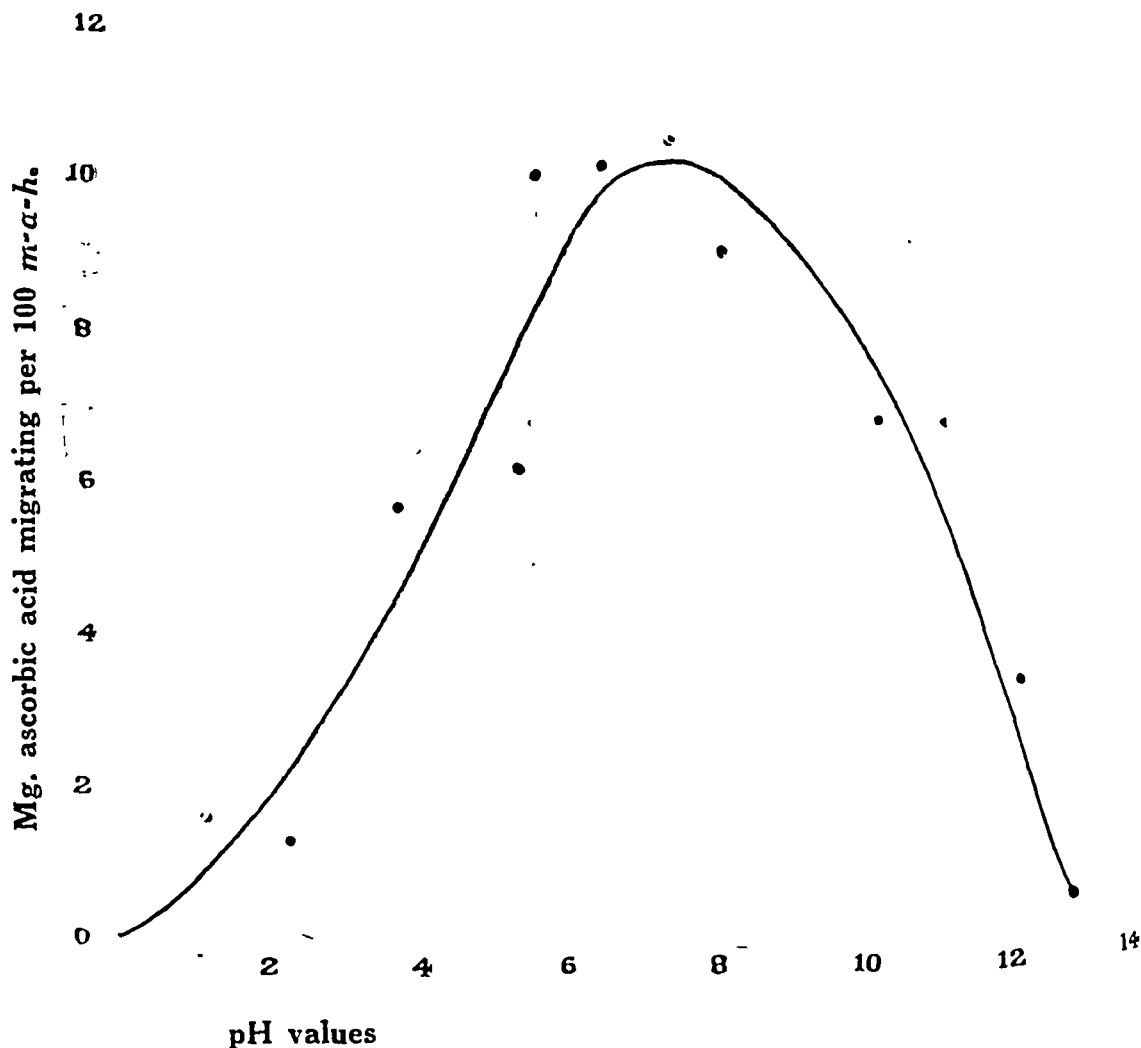


FIGURE 1. Showing the migration of ascorbic acid at various pH levels.

decline in the higher ranges. The shape of the curve might suggest that migration in the alkaline ranges decreases with increase of pH. But this is not so; the smaller amounts migrating are due to the greater destruction of ascorbic acid in the higher alkaline ranges.

In Figure 2, the amounts migrating, calculated as a percentage of the total amount of ascorbic acid present at the end of each experiment, are given. The results show that there is a progressive increase in migration up to a pH of about 7; at ranges above this level, the amount migrating remains stationary.

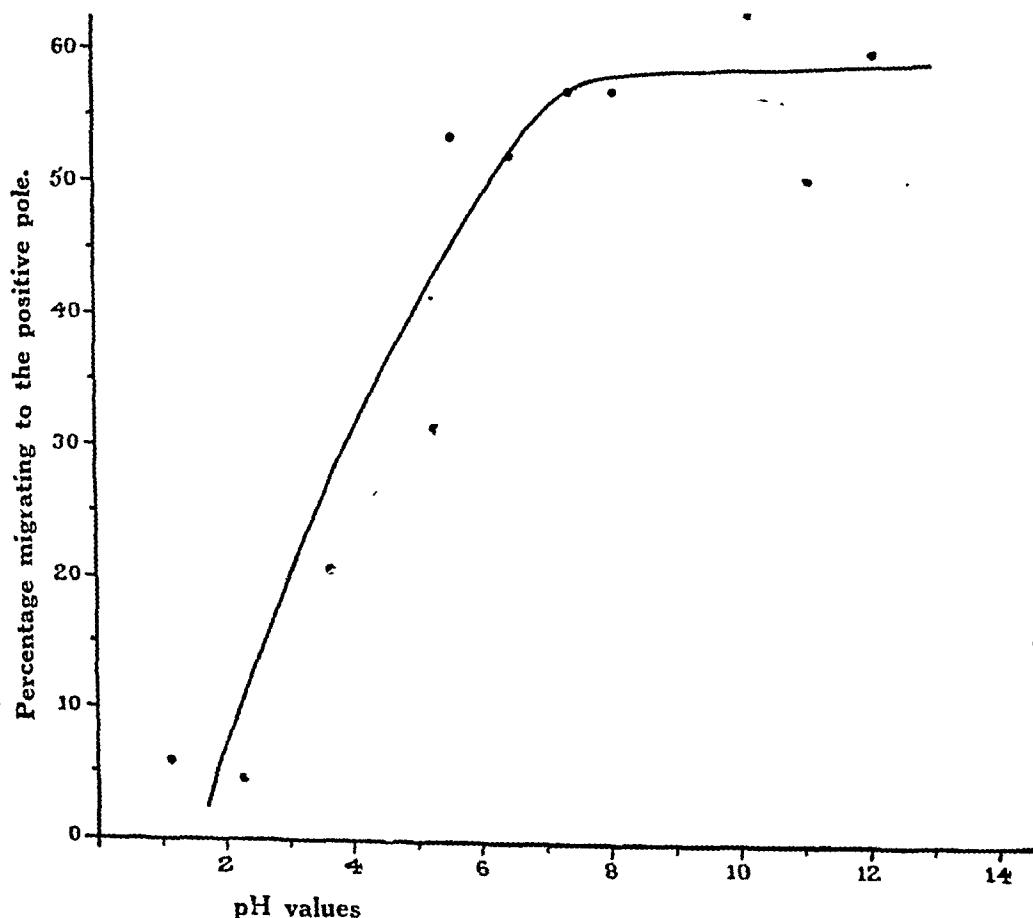


FIGURE 2. Showing the amounts migrating, calculated as a percentage of the total amount of ascorbic acid present at the end of each experiment.

Figure 3 represents graphically the influence of pH on the rate of destruction of ascorbic acid

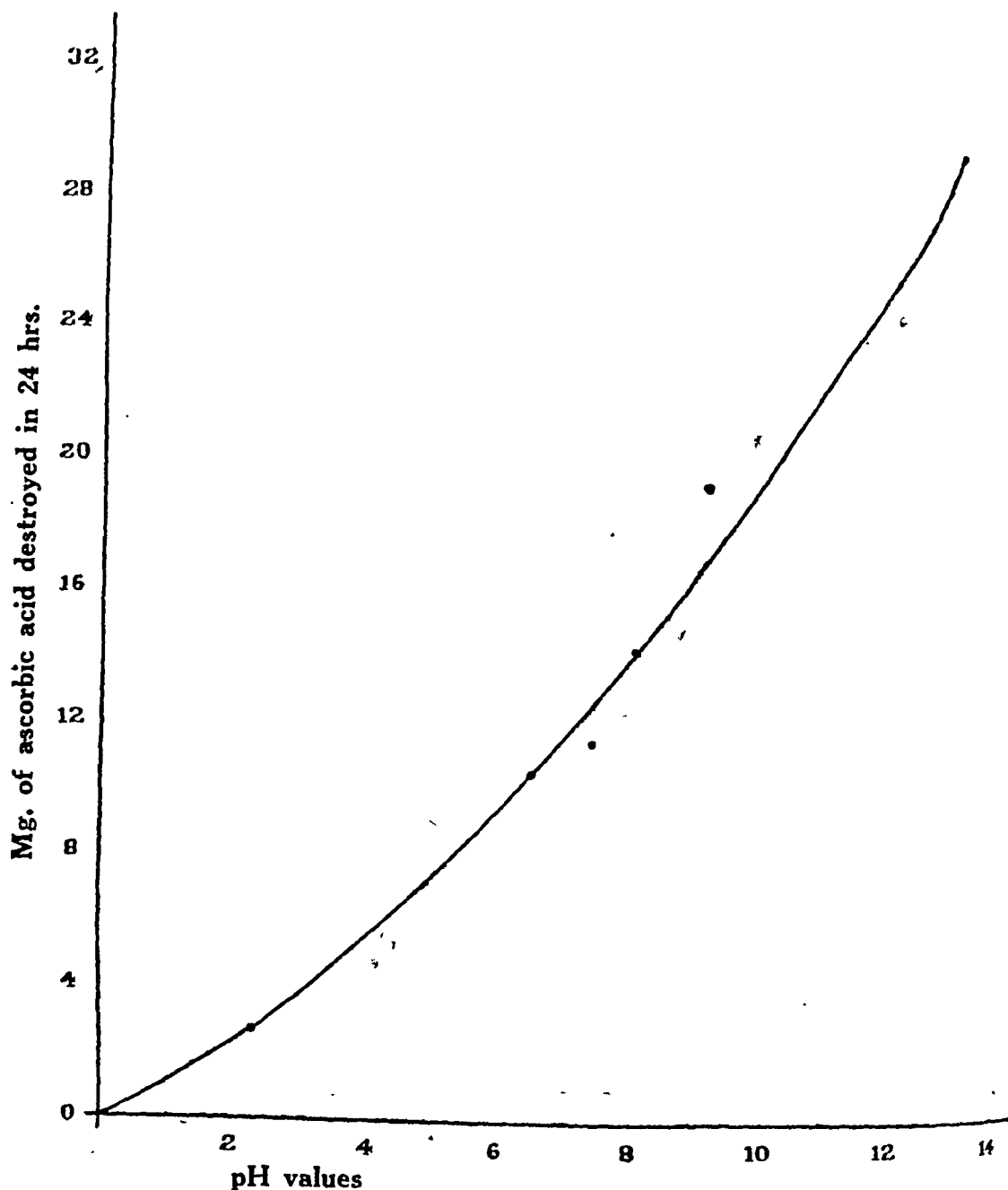


FIGURE 3. Showing the influence of pH on the rate of destruction of ascorbic acid.

DISCUSSION.

The evidence so far accumulated tends to show that ascorbic acid is a true acid, having a free carboxyl group, and migrating consistently at all pH ranges like any organic acid. But the idea of a free carboxyl group has been negatived by most

investigators working on the constitution of ascorbic acid, who on the other hand assign it a lactone structure. It seems difficult to explain the electrical migration properties at all pH ranges on the basis of a lactone structure. With a view to ascertaining if any other known lactone would migrate in an electrical field, similar experiments were repeated with phenolphthalein, a lactone, in solution in buffers containing about 20 per cent alcohol. Migration of phenolphthalein could be followed visually in alkaline ranges above pH 8.5, since the substance imparts an intense pink colour to the buffer. There was no migration of phenolphthalein to either side up to a pH of about 8.5, while on the alkaline side of this range it behaved as a true acid, migrating to the positive pole. This observation fits in with the known properties of lactones, viz., formation of an acid in an alkaline media. The results obtained with phenolphthalein seem to suggest that ascorbic acid does not behave like a lactone in solution, as there was migration even in the highly acid ranges and as there was no transition pH at which the lactone was split up, followed by migration to the positive chamber in the higher alkaline ranges, as was observed with phenolphthalein.

Again, Figure 2 clearly suggests that ascorbic acid behaves as a weak organic acid, showing comparatively lower migration figures in the acid ranges, due to the depression of ionization, and stationary migration in the alkaline ranges, indicative of complete ionization.

Herbert *et al.* (1933) have suggested that, though ascorbic acid does not contain a free carboxyl group, its acidic properties are due to an activated —CHOH group adjacent to a —CO— group, giving a reactive group of the type —C(OH) : C(OH)— . The question then arises whether organic compounds possessing a similar grouping, a —CHOH group adjacent to a —CO— group, would migrate in an electrical field. Fructose is known to contain this group and to possess acidic properties as well. To ascertain whether fructose would behave similarly to ascorbic acid in an electrical field, migration experiments were performed with fructose at pHs ranging from 1 to 13. In no instance was fructose found to migrate.

Titration curves as determined by the hydrogen electrode (Birch and Harris, 1933), and by the more refined glass electrode, give concordant results, and show that ascorbic acid in solution is a monocarboxylic acid. The present experiments have afforded evidence for the existence of a free carboxyl group in the molecule of ascorbic acid in solution. Perhaps all or most of the hitherto observed properties of ascorbic acid could be reconciled with this assumption.

SUMMARY.

1. The electrical migration of ascorbic acid was studied at various pH levels, ranging from 1.0 to 13.0. It was found to act as an electro-negative substance, migrating always to the positive pole.

2. The electrical migration at all pH levels shows it to be a true acid, with a free carboxyl group, and not a lactone, as phenolphthalein, a lactone, behaves differently in an electrical field.

3. The explanation of the acidic character of ascorbic acid, as being due to a —CHOH— group adjacent to a —CO— group, is not borne out, since fructose,

possessing a similar group, behaves differently to ascorbic acid in an electrical field.

4. The influence of pH on the rate of destruction of ascorbic acid was also studied.

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THE CHEMICAL NATURE OF VITAMIN B₁.

BY

G. NARASIMHAMURTHY, B.A., M.SC., A.T.C.

Parlakimedi Research Scholar.

(Nutrition Research Laboratories, I. R. F. A., Coonoor.)

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DURING recent years the problem of the chemical nature of vitamin B₁ has been extensively studied. Investigation of the absorption spectrum in the ultra-violet region (Heyroth and Loofbourow, 1934 ; Peters and Philpot, 1933 ; Smakula, 1934 ; Holiday, 1935) has shown that the molecule includes a pyrimidine structure to which a pyrrole ring is attached by a carbohydrate grouping. Further evidence in support of this structure is provided by the X-ray analyses of Bernal and Crowfoot (1933), whose researches helped to establish the identity of pure vitamin B₁ preparations from various laboratories, and by the chemical work of Windaus *et al.* (1934), Williams *et al.* (1935), Peters (1935), and Kinnersley *et al.* (1935). These latter investigations, purely chemical in nature, converge to show that the vitamin possesses a pyrimidine structure to which a thiozole ring is connected by a pentavalent N atom. Different methods of splitting the molecule were adopted, but there is general agreement that it includes labile groups one of which is an acid (a sulphonic acid). Chemical and physical investigations both suggest that vitamin B₁ belongs to the class of substances known as ampholytes.

A third line of attack, focussed on the ampholyte nature of the molecule, is opened up by physico-chemical methods. If the molecule includes a central basic group, with other groups attached to it, it should be possible, depending on the strength of the binding force, to discover the nature and number of these groups and locate their places in the molecule. Birch and Harris (1935) and Moggridge and Ogston (1935) studied the problem titrimetrically, using hydrogen as well as glass electrodes. The former workers deduced the existence of a basic group, with a pK value 4.9, the molecular weight of which, calculated from the equivalent of alkali used, was 330 ; and also of an acid group, pK 9.0. They point out the presence of a distinctive 'pseudo-acid' arrangement in the molecule. Moggridge

and Ogston obtained similar results except that they postulate a (more weakly) basic group at pK 3.4, and locate the basic group corresponding to that recorded by Birch and Harris at pK 4.8 instead of 4.9. According to these workers the pseudo-acid group is in the region of pH 9.0.

Sankaran and De (1934) investigated the problem by electrophoresis, using an acid clay adsorbate (the international standard preparation) as the source of vitamin B₁. Their results are not in agreement with those previously recorded, probably because they used the adsorbate instead of the vitamin B₁ itself. Birch and Guha (1931) and Ghosh and Guha (1935) obtained by electrical migration experiments a result more in conformity with that of other workers, showing that vitamin B₁ behaves as a base as far into the alkaline range as pH 8.5. Their study was not extended beyond a pH of 8.5.

The present paper records a re-investigation of the problem by electrophoresis, macro- and micro-, in which both chemical and biological tests for vitamin B₁ were used.

PREPARATION OF VITAMIN B₁.

Crystalline preparations of vitamin B₁ were made in the laboratories from brewer's top yeast, by the procedure described by Kinnersley and his co-workers



FIGURE 1. Photomicrograph
of vitamin B₁ crystals
($\times 120$).

(1933, 1935). Their purity was tested by physical, chemical and biological methods. The crystals obtained were transparent with a rectangular plate-like structure (see photomicrograph, Figure 1). The preparations were highly soluble in water and alcohol and showed the characteristic absorption band at $260m\mu$. On the addition of diazotized-sulphanilic acid, in the presence of a drop of formaldehyde, a pure pink tint was obtained; this was fairly stable for a period of 20 hours or more. The preparations were tested biologically on pigeons showing head retraction after being fed for 2 to 3 weeks on a diet of highly milled, washed, raw rice. One preparation produced cures in doses of 2.5γ , but the minimum curative dose of the preparation used in the experiments was about 15γ ; the presence of small quantities of inactive materials, along with the crystals, probably accounts for the fact that this

vitamin B₁ preparation was less potent biologically than others described in the literature.

ELECTROPHORESIS EXPERIMENTS.

Aqueous solutions, containing a drop of dilute hydrochloric acid to act as stabilizer, were made so that 1 c.c. of the solution contained 100 γ of the vitamin preparation. For each experiment at the various levels of pH, 3 c.c. of the solution (containing 300 γ) were used; to this was added 5 c.c. of either phosphate or glycocoll buffer solution of Sørensen (Clark, 1920). In two experiments at pH 11.0 and 12.0 respectively less concentrated solutions of vitamin B₁ had to be employed, owing to lack of sufficient material.

The electrophoresis apparatus used was that figured in the preceding paper by Ranganathan and Sankaran (page 214).

Vessels A, B and C are filled with the buffer solution to the extent shown in the diagram, the side tubes *c* and *b* being completely full. Chamber A, which is a short length of the bottom portion of a test-tube, is also initially filled with the buffer solution. Communication is established between the three chambers by opening the taps, and the levels equalized. Closing the taps, the vitamin solution is introduced into A, after a corresponding volume of buffer has been removed. The taps are then opened and a current from a D. C. source of 220 volts passed for 6 hours with non-polarizable electrodes.

The pH of the solutions in the various chambers was tested colorimetrically before and after the passage of the current. On the completion of the 6-hour period, the contents of the three chambers were brought to pH 3 and concentrated on a water-bath to a small bulk (5 c.c.). On removing the side tubes from the central chamber, an amount of solution roughly double that contained in the portion of the tubes dipping in the solution was returned to the central chamber; this precaution balances any loss of material from the central chamber which may occur as a result of diffusion or mechanical removal. The major part of each solution was stored in well-corked tubes in a fridaire for subsequent biological testing; the remainder was used for chemical analysis.

CHEMICAL ANALYSIS.

Chemical assay of vitamin B₁ depends on the formation of a suitable pink colour with diazotized-sulphanilic acid, the details of the method of investigation being described by Kinnersley *et al.* (1934). The quantity of vitamin B₁ present in test solutions is estimated by comparing the intensity of the pink colour with a mixture of Sørensen's acid and alkaline phosphates (2 and 8 vols. respectively), containing, in 103 c.c., 100 c.c. of phosphate mixture and 3 c.c. of 0.02 per cent phenol red. Equivalent quantities of the buffers were tested to eliminate the possibility of disturbing colour effects being produced by these alone; in no case was any colour observed. In mixtures containing glycocoll and vitamin B₁ a yellow tint in addition to the pink colour characteristic of the vitamin was observed on addition of the reagent. To allow for fading due to the presence of impurities, the intensity of colour was estimated after 30 minutes and again after 20 hours; actually very little fading was observed to take place. The results of the

chemical tests carried out 20 hours after the addition of the reagent are given in Table I :—

TABLE I.

Migration of vitamin B₁ in an electric field.

pH.	Original quantity of vitamin B ₁ (γ) preparation in central chamber.	Quantity (γ) migrating to positive pole.	Quantity (γ) migrating to negative pole.	Nature of charge.
5.2	300	0.0	75.0	+
8.0	300	0.0	100.0	+
9.2	300	0.0	32.0	+
9.8	300	41.0	21.0	± iso-electric region.
11.0	300	50.0	0.0	—
11.0	200	36.0	0.0	—
12.0	100	25.0	0.0	—
12.9	300	114.0	0.0	—

Strength of current = 2 milliamps, running for 6 hours at a P. D. of 220 volts D. C.

BIOLOGICAL EXPERIMENTS.

Healthy pigeons, between 210 grammes and 350 grammes in weight, were fed on washed milled raw rice. Within 15 to 28 days the majority developed 'polyneuritis'. Nearly all the birds used for assay showed head retraction in a varying degree.

Test materials were administered through a small catheter inserted in the œsophagus. The disappearance of head retraction within 2 hours indicated a positive test, experience having shown that improvement takes place within this period when an adequate dose of vitamin B₁ is given. Besides the head retraction

many of the birds had paresis of the legs and a considerable number showed green diarrhoea. In 'positive' cases the former usually disappeared concurrently with head retraction, but the diarrhoea often persisted.

Birds showing no improvement after 2 hours usually died within one or two days. Occasionally slight improvement after 2 hours was noted, but this was not maintained and subsequently the birds grew rapidly worse. In these cases a cure was sometimes obtained by re-enforcing the initial test dose. A number of birds which showed no response after 2 hours were given 100 mg. of the international vitamin B₁ standard to test their 'curability'. This produced a disappearance of the head retraction.

The results of these experiments are given in Table II :—

TABLE II.

Biological testing of solutions obtained by electrophoresis.

pH.	SOLUTION FROM POSITIVE POLE.			SOLUTION FROM NEGATIVE POLE.		
	Estimated amount of vitamin B ₁ in test dose (γ).	Birds treated.	Results.	Estimated amount of vitamin B ₁ in test dose (γ).	Birds treated.	Results.
5.2	0.0	2	— —	25.0	3	+ + +
8.0	0.0	2	— —	40.0	2	+ +
9.0	0.0	2	— —	{ 21.5 10.5	2	+ —
10.0	14.6	2	+ +	21.0	1	+
11.0	10.0	3	+ — —	0.0	2	+ —
11.0	36.0	1	—	0.0	3	+ — —
12.0	{ 12.0 25.0	1 1	+ +	0.0	1	—
12.0	20.0	1	—	0.0	1	—
12.9	40.0	2	— —	0.0	3	+ — —

+ = cure.

— = no cure.

The chemical tests described indicate that vitamin B₁ behaves as an electro-positive substance up to a pH of 9.0. At pH 10.0 migration is bi-polar. The

biological tests in solutions obtained from experiments in the acid range were clear-cut and satisfactory; beyond pH 10.0, however, they do not merit this description. Two negative pole solutions, shown by the chemical test to be devoid of vitamin B₁, produced 'cures', and on the positive side there was a lack of relation between the quantity of vitamin in the test dose and its curative effect. Failure to obtain 'cures' in certain cases with solutions from the positive pole in the alkaline ranges was probably due to giving insufficient amounts of the vitamin. No explanation can be offered of the two 'cures' produced by negative pole solutions at pH 11.0 and pH 12.9 respectively. In a general way, however, the biological tests confirm the chemical tests.

MICRO-CATAPHORESIS.

In order to throw further light on the problem, a series of micro-cataphoresis experiments were carried out.

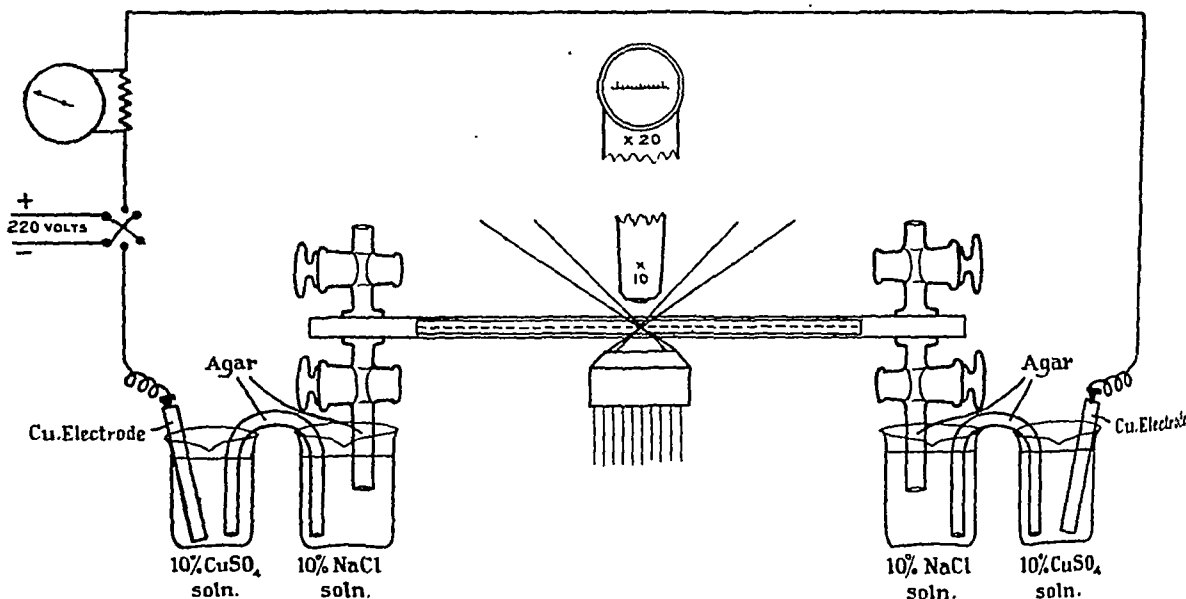


FIGURE 2. Arrangement of apparatus used for micro-cataphoresis experiments.

Abramson (1934) has found that the migration velocity of proteins in an electric field is similar to that of the same proteins in concentrated solution adsorbed on quartz particles; it might be inferred from this that quartz particles have no charge of their own. It was found, however, that a quartz suspension prepared according to the method described by Abramson displayed a negative charge, and that an alteration in the charge of quartz particles resulted from the presence of adsorbed vitamin B₁. Kieselguhr behaved similarly. In Abramson's work, the strong charge, carried by the proteins which were in high concentration, probably masked the charge of the quartz particles, whereas in the experiments described the vitamin B₁ was present in dilute solution. In the present work the micro-cataphoretic method of Abramson and his collaborators was used, with, however, the following difference in principle: the migration velocity of the carrier with and

without the presence of adsorbed vitamin B₁ was determined, and the difference between the two velocities was attributed to the nature and amount of the charge carried by the vitamin. In place of metallic electrodes used by other workers to lead the current through the solution under investigation (Brown and Broom, 1931, 1936) a non-polarizing arrangement (Figure 2) with an agar block separating the test solution from the electrodes was used. This excludes the possibility of the solution changing its pH as a result of the passage of the current.

EXPERIMENTAL.

Kieselguhr, which adsorbs vitamin B₁, was used in these experiments. It was washed with several changes of distilled water and finally a suitable distilled water suspension was made. One c.c. of the suspension was mixed with 0.5 c.c. of buffer solution and 1.0 c.c. of solution containing 10% of vitamin B₁ crystals. One c.c. of distilled water was added to control solutions in place of the vitamin B₁ solution. The solutions, after standing for 5 minutes, were put in the micro-cataphoretic cell [the cell of Brown and Broom (1931) modified by Millwood] and the migration of particles moving midway between the roof and floor of the cell was observed under darkground illumination. The particular cell used in this work is 16 cm. long, 0.78 mm. deep and 5 mm. wide. The current used was 220 volts D. C., its strength being measured by a galvanometer and shunt included in the circuit. The time taken by a particle, chosen at random, to cover a distance of 1 mm. was determined by means of a micrometer scale in the eye-piece. Velocities were also measured with the current reversed. Table III gives the observed velocities at different levels of pH, each figure representing the mean value of 10 to 20 observations with both directions of current :—

TABLE III.

(A) *Migration velocity of Kieselguhr particles alone.*

pH.	Current strength in m. a.	Average time in seconds taken by Kieselguhr particles <i>without</i> adsorbed B ₁ to cover 1 mm.	Calculated time in seconds taken per m. a., per mm.	Migration velocity per 1 m. a. [mm. per sec.].	Nature of charge.
5.9	0.66	2.2	1.45	0.685	Negative.
8.0	1.00	1.7	1.70	0.600	"
8.9	1.33	2.05	2.76	0.362	"
10.0	1.08	3.00	3.24	0.308	"
11.0	1.08	3.00	3.24	0.308	"
12.0	1.16	2.80	3.24	0.308	"

TABLE III—*concl'd.**(B) Migration velocity of Kieselguhr particles with adsorbed vitamin B₁.*

pH.	Current strength in m. a.	Average time in seconds taken by Kieselguhr particles <i>with</i> adsorbed B ₁ to cover 1 mm.	Calculated time in seconds taken per m. a., per mm.	Migration velocity per 1 m. a. [mm. per sec.].	Nature of charge.
5.9	0.83	10.5	8.72	0.114	Negative.
6.0	1.08	2.2	2.38	0.42	„
8.9	1.33	6.2	8.246	0.121	„
10.0	1.00	2.6	2.6	0.384	„
11.0	1.16	2.0	2.32	0.43	„
12.0	1.16	2.3	2.67	0.374	„

In Table IV the differences between the migration velocities of the particles in the presence and absence of the vitamin are calculated :—

TABLE IV.

Difference in migration velocities of Kieselguhr particles caused by adsorbed vitamin B₁.

pH.	Difference.	Nature of charge.
5.9	0.571	Positive.
8.0	0.180	„
8.9	0.241	„
10.0	0.072	Negative.
11.0	0.092	„
12.0	0.048	„

Since the concentration of vitamin at various pH levels was the same, it is assumed that the differences in velocity were due to the charge of the vitamin. In Figure 3 the data given in Table IV are shown graphically :—

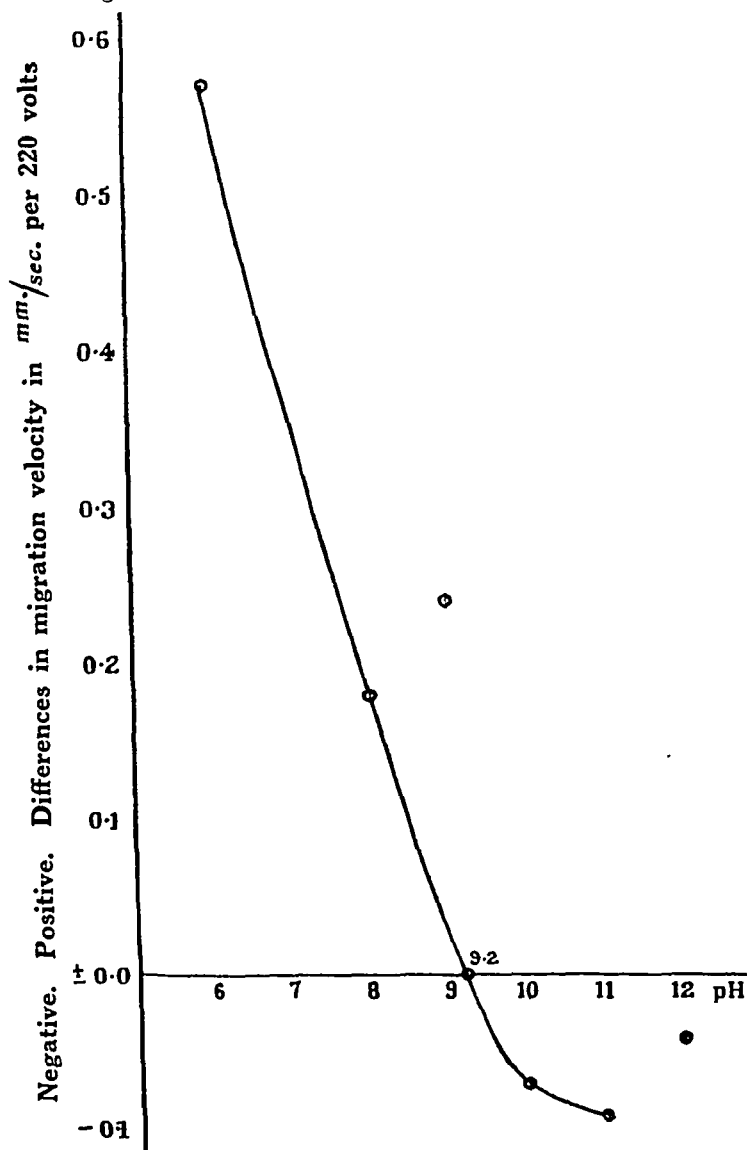


FIGURE 3. Differences in migration velocity of Kieselguhr particles at different levels of pH.

A further indication of the position of the iso-electric point is provided by the current strength carried by the solutions at each level of pH with and without adsorbed vitamin. Theoretically the vitamin possesses minimum potential at the

iso-electric point, and it seems justifiable to assume that the difference between the conductivity of the Kieselguhr suspension alone and that of the suspension containing vitamin B₁ will be least at this point. Galvanometer readings at each hydron level, and the differences between the two sets of readings, are given in Table V. In Figure 4 the difference is displayed graphically. It will be observed that the

TABLE V.

Strength of current conducted by Kieselguhr suspension with and without adsorbed vitamin B₁.

pH.	Galvanometer reading with vitamin (m. a.).	Galvanometer reading without vitamin (m. a.).	Difference (m. a.).
5.9	0.83	0.66	0.17
8.9	1.08	1.00	0.08
8.9	1.33	1.33	0.00
10.0	1.00	1.00	0.00
11.0	1.25	1.08	0.17
12.0	1.25	1.16	0.09

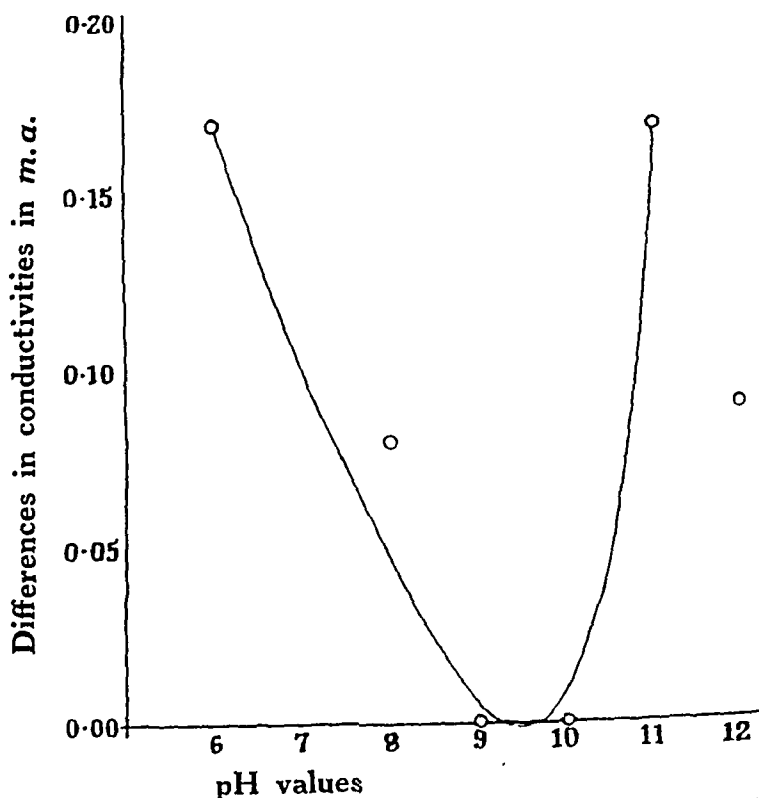


FIGURE 4. Differences in conductivities of solutions at different levels of pH.

difference is minimum between pH 9.0 and pH 10.0 and that this series of observations, while they cannot sharply define the iso-electric point, are in line with the rest.

The results of the micro-cataphoresis experiments suggest that the iso-electric point lies between pH 9.0 and pH 10.0 and nearer pH 9.0 than pH 10.0.

SUMMARY.

The problem of the iso-electric point of vitamin B₁ was investigated by electrophoresis experiments and an attempt was made to confirm the findings by biological tests. The same problem was also studied by micro-cataphoresis. The experiments described converge to show that the iso-electric point lies between pH 9.0 and pH 10.0, nearer the former than the latter.

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IMPORTANCE OF PHYTOSTERYL ACETATE TEST IN DETECTING HYDROGENATED FATS IN GHEE.

BY

RAI BAHADUR K. N. BAGCHI, B.Sc., M.B., D.T.M., F.I.C.,
Chemical Examiner to the Government of Bengal, Calcutta,

AND

N. S. MAZUMDAR, B.Sc.

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THE phytosteryl acetate test is the only reliable test for detection of vegetable fats in ghee and is considered to be the 'final court of appeal' (Bolton and Revis, 1913). The reliability of this test is seriously affected if a small quantity of paraffin wax is added to a mixture of animal and vegetable fats and this fact is taken advantage of by those whose interest is to sophisticate butter or ghee for commercial purposes. The influence of paraffin wax on the melting point of phytosteryl acetate crystals is remarkable. The presence of as small a quantity as 0.02 per cent interferes with the test by lowering the melting point of the crystals much below 100°C. and thus renders the test absolutely useless. Polenske devised (Lewkowitsch, 1921a), however, a method for detecting paraffin in butter and also removing it from the mixture before the actual test is applied.

Lately, another danger in the form of 'vegetable ghee' has appeared on the scene and has already been proved to be a potent factor in upsetting this important test. Vegetable ghee is a mixture of hydrogenated vegetable oils and is extensively used nowadays as an adulterant of ghee and its detection is therefore of utmost importance to the public analysts. During hydrogenation of the vegetable oils at high temperature (250°C.) the phytosterols are completely reduced to resinous hydrocarbons (quoted by Godbole and Sadgopal, 1930) and the characteristic crystals of these sterols are not formed. The hydrocarbons thus produced do not form the sterides with digitonin and on acetylation they produce no acetates. But if the test is properly carried out and every step is carefully watched, certain changes are noticed which furnish a clue for identification of hydrogenated vegetable fats in ghee.

There are two important methods for carrying out this test:—

- (1) the usual saponification and ether extraction method, and
- (2) the digitonin method as described by Klostermann (1913). The second method is very simple and less laborious but more expensive than the first one which takes longer time and sometimes produces troublesome emulsions 'on the slightest provocation' (Elsdon, 1926). But in spite of these drawbacks the saponification method is adopted for detection of hydrogenated fats. The digitonin method is not suitable for this purpose as digitonin combines with only the sterols to form the digitonin sterides and has got no action on the hydrocarbons, the detection of which forms the basis for this investigation. The saponification method as described in some of the standard books is vague and does not help much. A correct method has been described in this paper in detail and if it is followed carefully the result will be quite satisfactory and no emulsion will be formed to spoil the experiment.

(1) About 100 grammes of adulterated ghee are taken in a litre flask and about 200 c.c. (double the amount of ghee) of 2 N alcoholic potash [almost colourless and prepared from KOH (Merck's G. R.) and Merck's absolute alcohol] are added and saponified on a water-bath under a reflux condenser for about three hours. Instead of taking Merck's alcohol, ordinary Indian-made absolute alcohol may be used after purifying it by treatment with KOH and silver nitrate as described by Lewkowitsch (1921b). After saponification the soap is cooled, transferred to a two-litre separator and diluted with about 800 c.c. of water. The level of the soap solution on the separator is marked with a grease pencil. Three hundred c.c. of ether are now added and the separator is very gently tilted up and down for about half a dozen times—shaking at this stage is to be strictly avoided as it results in forming an emulsion. The ether is allowed to separate. If the separation is not fairly quick and a fluffy layer appears just below a thin layer of ether a few drops of alcohol would hasten the separation and about 200 c.c. of ether would be separated—the remaining 100 c.c. being taken up by the soap solution to form a harmless emulsion and is indicated by the pencil mark. The separated ether is transferred to a litre flask. The second extraction with another lot of 300 c.c. of ether is made but this time with a gentle shaking for about half a minute. It is extracted for the third time by shaking gently for about five minutes with 200 c.c. of ether. The fourth extraction by shaking vigorously with 200 c.c. of ether is made but the shaking is continued intermittently for about 15 minutes. If after any of these extractions the ether does not separate and appears to form an emulsion—a crisis which is not likely in this method, may be got over by diluting the soap solution with 200 c.c. to 300 c.c. of water and adding more ether (about 200 c.c.) and tilting up and down as was done in the beginning and the extraction is proceeded with very cautiously avoiding shaking as far as possible and completing the extraction in five or six stages.

(2) The combined ether extracts are distilled and the ether is recovered for subsequent extractions. The residue in the flask consisting of some unsaponified fat, soap, alcohol, water, all the sterols and other unsaponifiable matters including the resinous hydrocarbons, is again saponified with 20 c.c. of the same alcoholic potash under a reflux condenser for about an hour. It is then cooled and diluted

with about 100 c.c. of water and the soap solution is transferred to a smaller separator (about 300 c.c.) and extracted with 100 c.c. of ether with gentle shaking for about two minutes. The ether is separated and transferred to another separator. The extraction is repeated twice with 50 c.c. of ether with gentle shaking and twice more with 50 c.c. of ether with vigorous shaking. The combined ether extracts are washed thrice with about 30 c.c. of water each time. The washed ether containing the sterols and the hydrocarbons with traces of water is filtered through a dry filter-paper into an Erlenmeyer flask and distilled to dryness when the following changes are to be observed in the residue: (a) a light brown mass is obtained with light or deep brown droplets of a sticky resinous hydrocarbon which appears to be the characteristic feature of the presence of hydrogenated fats, (b) these droplets readily dissolve in hot alcohol but reappear on evaporating and cooling, (c) if the amount of hydrogenated fat is small only a sticky, brown substance is found at the bottom of the flask. In some hydrogenated fats, e.g., in Cocogem, the droplets are not deep brown but yellowish in colour resembling oil globules. If the amount of hydrogenated fats is 20 per cent or less, the residue becomes crystalline and the droplets are smaller and fewer. If on the other hand it exceeds 25 per cent the residue becomes amorphous and sticky and the droplets are larger in size and in number.

(3) The residue is now dissolved in about 5 c.c. of absolute alcohol on a water-bath and is transferred to a small glass evaporating basin. The flask is rinsed twice with about 2 c.c. of alcohol and the washings are transferred to the basin. On evaporating, the crystals of cholesterol begin to appear—some floating as a thin film on the surface and some as a white deposit. The following facts are to be noted at this stage: (a) the deposit is not perfectly crystalline and is slightly tinged brown, (b) the mother liquor is also brown and turbid and not clear as in the case of pure ghee, (c) under the microscope the cholesterol crystals will be found but no phytosterol crystals nor the mixed forms (Lewkowitsch, 1921c), (d) a colourless amorphous deposit is found scattered all over the field and in highly adulterated samples, this amorphous deposit forms the main bulk in the field of the microscope and the detection of the cholesterol crystals becomes comparatively difficult, and (e) on evaporating the contents of the basin to dryness, the brown droplets re-appear.

(4) The contents of the basin are now evaporated to dryness on a water-bath and about 3 c.c. (3 c.c. to 5 c.c.) of pure acetic anhydride are added and the basin is covered with a watch-glass and gently heated on a wire gauge over a small flame to boiling and in a few minutes the residue is dissolved and acetylated. If it does not dissolve readily, little more acetic anhydride may be added. The basin is then transferred to a water-bath and heating is continued to expel the excess of anhydride—the watch-glass being removed to hasten evaporation. When there are only a few drops left in the basin, it is cooled and on cooling the sterol acetates set to a brown mass. About 5 c.c. of 95 per cent alcohol are now added. It is warmed and allowed to evaporate and crystallize. The crystals (cholesteryl acetate and also of phytosteryl acetate if present) are filtered through a small filter (diameter of the funnel is about 1") moistened with alcohol and washed twice with 1 c.c. of cold 95 per cent alcohol. The following points are to be noted at this stage: (a) the colour of the mother liquor is distinctly brown, (b) the resinous hydrocarbons are seldom seen as brown droplets at this stage but appear as a sticky, brown coating at the bottom of the basin and cannot be detached from it, this stickiness being

characteristic and easily felt with the finger tip. In pure ghee no such changes take place and the crystals are not sticky and can be easily and completely separated from the basin, (c) if the mother liquor, after separation of the crystals by filtration, is evaporated to dryness and the melting point of the dry residue (a mixture containing some resinous hydrocarbons, normal hydrocarbons of ghee and some cholesteryl acetate) is determined, it will be found to vary from 50°C. to 70°C. or even lower, and (d) in pure ghee this residue is a pale yellow liquid at the room temperature and solidifies on cooling.

(5) A few crystals from the filter (the first crop) are taken out with the point of a knife and dried on a hot porous plate and its melting point is determined. The remaining portion of the crystals is transferred completely from the filter to the basin and dissolved again in 5 c.c. of 95 per cent alcohol and allowed to crystallize. The crystals (the second crop) are filtered and washed as before and some of them are taken out and dried for determination of the melting point. By repeating this process five crops of crystals can be easily isolated and their melting point determined. The melting point of the first crop of crystals is about 113°C. in cases of pure ghee but in samples containing hydrogenated fats it may come down to 108°C. (usually 110°C.). The lowering of melting point of the first and possibly of the second crop of crystals is due to presence of the hydrocarbons of low melting point formed during hydrogenation and is therefore of importance for detection of hydrogenated fats in ghee.

In cases of adulteration of ghee with ordinary vegetable fats the steady increase in the melting point of the successive crops of crystals conclusively prove the presence of vegetable oils and fats. But if it is adulterated with hydrogenated vegetable fats no such increase in the melting point is observed—practically the same melting point in every crop of crystals (from the third to the fifth) is obtained, indicating the presence of only the cholesterol of ghee. The appearance of a brown, sticky and resinous substance at the various stages of the experiment is therefore an unmistakable proof of the presence of hydrogenated fats. If the experiment is performed strictly according to the method described above, the presence of 10 per cent hydrogenated fats will be detected without much difficulty. In order to be thoroughly conversant with this method it is advisable to experiment with one or two samples of pure ghee and pure vegetable oils (unhydrogenated) before the actual experiment is performed with adulterated samples, otherwise the phenomena characteristic of hydrogenated fats are likely to be missed. Experiments with Cocogem and two different brands of vegetable ghee (made in Holland) mixed with pure ghee in different proportions varying from 10 per cent to 66 per cent indicated the presence of the hydrogenated fats in all the mixtures. It is stated that hydrogenated fish oils are now imported from Japan for use as an adulterant of ghee. As a genuine sample of hydrogenated fish oil was not available and could not be analysed we are not in a position to state the actual changes that are likely to be met with at different stages of the experiment but we are sure that this test will also detect its presence in ghee because the cholesterol of fish oil is more easily reduced to resinous hydrocarbons even at a lower temperature. It may, therefore, be said that the phytosteryl acetate test still holds the ground and is equally useful for detection of all forms of hydrogenated fats and oils in ghee.

It may be mentioned in this connection that the quantity of ghee (100 grammes) usually recommended for the phytosteryl acetate test is one of the reasons which

stand in the way of detecting vegetable fats and oils in ghee by this test as the formal samples generally received by the public analysts contain about 50 grammes or even less. But a little experience in the technique described in this paper will enable them to take up and successfully analyse 25 grammes of moderately adulterated ghee and give a definite opinion one way or the other.

SUMMARY.

The technique of the phylosteryl acetate test has been described in detail. The following indications are important for detection of hydrogenated fats: (1) Formation of brown droplets of a resinous substance on evaporating and cooling the hot alcoholic solution of the resaponified sample or its ether extract. (2) Under the microscope the typical cholesterol crystals partially covered with an amorphous deposit are seen but no phytosterol crystals nor the intermediate forms. (3) On acetylation of the ether extract (sterols and other unsaponifiable matters) the resinous substance forms a sticky, brown coating which cannot be detached from the basin. (4) After acetylation, the residue (sterol acetate and hydrocarbons) is allowed to crystallize from alcohol and five different crops of crystals are obtained. The melting point of the first fraction and possibly of the second is about 110°C. and that of the last three fractions is constant at about 114°C. which is the melting point of cholesteryl acetate. (5) The first filtrate is evaporated to dryness and the residue gives a low and indefinite melting point—varying from 50°C. to 70°C., while in pure ghee the residue is a pale yellow, oily liquid at room temperature and the droplets of the brown and sticky hydrocarbons are not noticed. The digitonin method is quite satisfactory for detection of ordinary vegetable fats in ghee but not so for hydrogenated fats.

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SOME OBSERVATIONS ON THE INDOPHENOL (2 : 6 DICHLOROPHENOL-INDOPHENOL) REDUCING PROPERTIES OF URINE.

BY

BREVET-COLONEL R. N. CHOPRA, M.A., M.D. (Cantab.), M.R.C.P. (Lond.),
C.I.E., K.H.P., I.M.S.,

AND

A. C. ROY, M.Sc.

Indigenous Drugs Inquiry, Indian Research Fund Association.

(From the School of Tropical Medicine, Calcutta.)

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SINCE the introduction of the method for the titration of vitamin C against 'red ox' dye (indophenol) by Tillmans in 1932, the method has been used by a number of workers. Its application has led to the discovery of the presence of this vitamin in fairly high concentrations in a number of unexpected sites in the animal body, such as the medulla and the cortex of the suprarenal gland, the aqueous and vitreous humours, and the wall of the gut and also the urine. But the evidence put forward to establish the identity of such substance with ascorbic acid can not be said to be quite convincing, at least so far as the urine of normal individuals and other body fluids and tissues are concerned.

Birch and Dann (1934) applied the silver nitrate staining test to a number of tissues which have been found by indophenol titration to contain ascorbic acid, with results which were not always in harmony with those obtained by the latter method. Some tissues found by the titration method to contain ascorbic acid, gave little or no staining with silver nitrate but they thought that their results supported the conclusion formulated by Harris and Roy (1933), viz., 'that a positive result with this test indicated the presence of ascorbic acid but a negative result did not necessarily mean that ascorbic acid was absent'. But this leaves the question as to the criterion for testing the presence or absence of ascorbic acid still unsettled.

Biological tests (the recovery test of Harris *et al.*, 1932) carried out by Birch and Dann to verify the results obtained by the titration method with the eye lens and aqueous humour of the ox (which were found to have appreciable indophenol reducing properties) were unsuccessful and gave no indication of the presence of ascorbic acid. According to these authors the failure of the test suggested not so

much the absence of ascorbic acid in the lens, as the presence of some other substance exerting a positive toxic effect. Another biological test was however tried, with alterations considered likely to diminish the effect of toxicity of the lens, viz., the tooth structure method of Höjer as modified by Key and Elphick (1931) and by this method, according to them, undoubted evidence of the presence of ascorbic acid in the lens of the ox's eye was obtained.

In these experiments three of the four animals receiving a supplement of the lens died before the first of the negative control animals, the average time between the commencement of the experimental period and death being $4\frac{1}{2}$ days for those receiving the dried lens and $6\frac{1}{2}$ days for the negative controls. The animals receiving aqueous humour lived through the experimental period of nine days but did not show any increase of weight as did the positive controls receiving 3 c.c. of orange juice daily. It should be noted that the detoxicated specimens of the lens were not tested by the curative method.

It is difficult to see how the same specimens administered in the same way (viz., by the mouth) could produce different results with different methods of testing and moreover how it could produce one effect of vitamin C (viz., protection against scurvy) and not the other (viz., increase in weight).

As to the question of the presence and amount of this vitamin in the urine also, conflicting results are reported. Van der Walle (1922) reported that a daily dose of 20 c.c. of fresh normal urine afforded no significant protection from scurvy to guinea-pigs on a scorbutic diet. Harris and Roy (1935) suggested an explanation for this failure on the ground that either the urine was not fed in a sufficiently large dose or it was not fed soon enough after it had been passed, to guard against inactivation of the urine.

Van Eekelen and his co-workers (1933) showed that the indophenol reducing capacity of human urine increased after the consumption of much fruit or of high doses of decitrated lemon juice.

Harris, Roy and Ward (1933) showed that in a number of individuals, the daily output of vitamin C, as measured by the indophenol titration, was surprisingly constant, generally in the neighbourhood of 30 mg. to 33 mg. per day. They also showed that if a normal individual is given a single very large dose of vitamin C, the urinary output of ascorbic acid (indophenol titration) sharply rises, reaching a maximum in about three hours, it then rapidly drops again and within a day or so, reached the normal resting level of about 33 mg. per day, where it remained remarkably steady although the subject may be restricted for a week or more to a vitamin-C free diet. From the results obtained by these workers it is evident that the 'normal' daily loss of about 33 mg. is somewhat greater than the reputed minimum daily requirement for man (viz., 1 oz. of orange or lemon juice containing about 20 mg. of ascorbic acid).

It is somewhat surprising that the system should be so wasteful regarding a substance the absence or diminution of which would involve it in serious complications. In the ordinary course of things, the body excretes those substances, the presence of which in excessive amount would be deleterious to it but no cases of hypervitaminosis C are recorded.

These workers further showed that the urinary output depended both on the immediate vitamin intake and also on the past nutritional history or in other words.

on the state of vitamin saturation and they have suggested the application of this titration technique as a diagnostic test for detecting states of vitamin-C sub-nutrition in human beings.

Harris and Roy (1935) adduce as a proof for the specificity of the titration method the following: the virtual disappearance of the titre in scurvy and its restoration when vitamin C is fed and the proportionality between the titre and the amount of vitamin C in the diet. But from the results obtained by us, to be described later, it will appear that the indophenol titre of the urine is much reduced in other conditions also (*viz.*, epidemic dropsy), without any apparent manifestation of the symptoms of scurvy. Hess and Benjamin (1934) on the other hand have concluded from their experiments on children that under ordinary dietary conditions, the amount of vitamin C present in human urine is so small as to be considered negligible.

Recently, however, Johnson and Zilva (1934) by the daily administration of urine containing 0.5 mg. ascorbic acid (titration method) to guinea-pigs which had subsisted for 14 days on a scorbutic diet found that the anti-scorbutic potency of the urine dose was of the same order as that of 0.5 mg. of ascorbic acid. It must, however, be noted in this connection that the urines used for these biological tests were those of persons who were dosed with large amounts of ascorbic acid (875 mg. and 1,000 mg.) before the urine was collected. These experiments do not prove the excretion of vitamin C through the urine of normal individuals under ordinary dietary conditions.

In the same paper the following observations of these workers are also worthy of note. 'Three of the four experimental subjects while using ordinary mixed diet on which they usually subsist, passed, during the winter months, urine, which for days scarcely reduced indophenol. Even when the titrations were significant, the output of ascorbic acid per 24 hours was of the order of 10 mg. to 15 mg. During the summer the indophenol reducing capacity rose to some extent. The fourth subject used a diet in which bread and potatoes were replaced by green vegetables and in addition he consumed regularly two oranges a day. In consequence his normal urinary output of ascorbic acid was 80 mg. to 150 mg. per day even during the winter. At no time in the period of 8 months, during which the experiments were carried out, were there any indications even of latent scurvy in any of the four subjects'. This is in accord with our own findings. One of us regularly excreted urine, the reducing properties of which were very small yet no apparent manifestation of the symptoms of scurvy could be detected.

The main points which emerge from all these observations are:—

1. Some of the tissues and body fluids have the property of reducing indophenol to an appreciable extent.
2. That in normal human urine, a substance is excreted in variable amounts which has this property.
3. This substance reduces indophenol in the same manner as ascorbic acid.
4. If a person is given a large dose of ascorbic acid containing substances, the indophenol reducing property of the urine is considerably increased for some time.
5. The biological tests give contradictory results and cannot be said to be entirely satisfactory.

So that it appears that the proof of the existence of ascorbic acid in the urine and in other tissue fluids excepting perhaps suprarenal cortex has not been established beyond the possibility of doubt. 2:6 dichlorophenol-indophenol is one of the indicator dyes, and it is very readily reduced. In fact it almost tops the list of such dyes in this respect. It is, therefore, quite conceivable that some other substances present in the urine and tissues are responsible for the reduction of the dye.

So far as the actual method of titration is concerned it is not always possible to titrate the urine immediately after it is passed. This difficulty is especially felt with respect to the hospital patients and with night specimens. Therefore it is necessary that a suitable preservative should be used which will keep the indophenol titre unchanged for at least 18 to 20 hours. The addition of 5 per cent trichloroacetic acid was first recommended. Later on, Harris and Roy (1935) preferred the addition of 10 per cent of glacial acetic acid and remarked that under such conditions the vitamin C in the urine may be preserved for about 10 to 12 hours with relatively little loss. Again, Johnson and Zilva (*loc. cit.*) used sulphuric acid as a preservative. They collected the urine over sulphuric acid (strength or quantity not mentioned). Under these conditions the ascorbic acid was found by them to be stable for several days. Titrations were carried on at pH 3. Acetic acid in their opinion had a less stabilizing effect.

It was, therefore, our object to determine if there was any difference in the titre under the above conditions of titration and under which of the above conditions the urine is preserved more efficiently. Because a part at least of the urine passed during 24 hours must necessarily be preserved for some hours, and, if the preservative is not efficient, that would make the titration values obtained with regard to the 24 hours' specimen relatively of little consequence.

In our experiments urines were usually collected 4 hours after the midday meal.

TABLE I.

Nature of the specimen.	REDUCING PROPERTIES CALCULATED IN TERMS OF MG. OF ASCORBIC ACID PER 100 C.C.			
	TRICHLOROACETIC ACID 5 PER CENT.		GLACIAL ACETIC ACID 10 PER CENT.	
	Immediate.	3 hours after.	Immediate.	3 hours after.
1. Normal (A. C. R.)	6.6	6.25	5.0	3.4
2. do. "	6.25	..	5.4	..
3. do. "	2.85	1.8	2.2	1.2
4. A portion of the above specimen was kept without the addition of either CCl ₃ COOH or glacial acetic acid and then titrated after 3 hours as usual.	..	2.5	..	1.4
5. Normal (A. C. R.)	4.5	4.1	3.40	1.80
6. do. "	5.0	..	3.40	..
7. do. "	5.0	..	3.2	..

TABLE I—concl'd.

Nature of the specimen.	REDUCING PROPERTIES CALCULATED IN TERMS OF MG. OF ASCORBIC ACID PER 100 C.C.			
	TRICHLORACETIC ACID 5 PER CENT.		GLACIAL ACETIC ACID 10 PER CENT.	
	Immediate.	3 hours after.	Immediate.	3 hours after.
8. Normal (A. C. R.)	5.0	..	3.5	..
9. do. (B. C. D.)	4.0	..	3.0	..
10. A portion of the above urine was boiled for 5 minutes, cooled, made up to original volume and then 10 per cent glacial acetic acid added.	..	..	2.4	..
11. Normal (B. C. D.)	4.0	4.0	2.8	2.0
12. do. (A. C. R.)	2.0	2.0	..	..
13. do. „	3.7	3.7	..	..
14. Suffering from acute abdominal pain (H. C. M.).	2.0	..	1.25	..
15. Polyarthritis; takes moderate amount of meat every day.	5.4	..	4.4	..
16. do.	6.2	..	4.8	..
17. Gout (before atophan)	7.7	5.9	7.0	2.0
18. do. after 9 tablets of atophan ..	6.2	..	3.3	..
19. Cured E. D.; takes moderate amount of meat, brown bread and fruits.	14.2	..	16.0	..
20. Cured E. D.	16.6	16.6	18.5	13.8
21. E. D. cured	14.2	10.0 (5 hours).	12.5	3.3 (5 hours).

E. D. = Epidemic dropsy case.

TABLE II.

Nature of specimen.	REDUCING PROPERTIES IN TERMS OF MG. OF ASCORBIC ACID PER 100 C.C.								
	TRICHLORACETIC ACID 5 PER CENT.			ACETIC ACID 10 PER CENT.			H ₂ SO ₄ (pH 3).		
	Immediate.	3 hours after.	21 hours after.	Immediate.	3 hours after.	21 hours after.	Immediate.	3 hours after.	21 hours after.
1. Normal (A. C. R.)	5.7	5.2	5.5	4.1	3.7	2.8	4.3	3.8	3.25
2. do. „ ..	6.6	..	6.0	4.5	..	2.8	4.5	..	4.10
3. Normal (K. S. M.)	7.2	..	6.6 (5.5) 3 weeks after.	4.4	..	3.2 (1 mg.) 3 weeks after.	4.1	..	3.7 (1.0) after 3 weeks.

From Tables I and II it would appear that :—

1. Even normal individuals excrete variable amounts of the reducing substances through the urine.
2. Their concentration in the urine depends considerably on the diet.
3. The titres by the acetic acid method yield consistently lower values than those obtained by the trichloroacetic method.
4. None of the substances used can be said to be an efficient preservative to guard against inactivation due to storage but if we are to choose, CCl_3COOH seems to be more effective than the others in this respect.

To see whether there is any difference in the titre when different acids of varying concentrations were used, the tests shown in Table III were carried out. For each set, the same specimen of urine was used :—

TABLE III.

Nature of specimen.	CCl_3COOH 5 per cent.	IN TERMS OF MG. OF ASCORBIC ACID PER 100 C.C.; 1 C.C. OF THE ACIDS ADDED TO THE INDICATOR.					
		10 per cent acetic acid.	Glacial acetic acid.	50 per cent H_2SO_4 .	Con. HCl.	HCl. 1 in 4.	20 per cent CCl_3COOH .
Normal (A. C. R.)	5.0	3.3	3.3	10.0	40.0	8.0	8.0
0.5 c.c. of 2N acids added to the indicator.							
	CCl_3COOH 5 per cent.	10 per cent acetic acid.	Acetic acid.	Oxalic acid.	HCl.	H_2SO_4 .	
do. „ ..	5.0	3.4	3.0	4.5	6.6	6.6	

These results show that the titre varies considerably with the nature and concentration of the acids.

Some time ago Ahmed (unpublished) made an important observation that the indophenol reducing properties of urine were increased on a high protein diet and that the nitrogen content of the urine and its indophenol reducing capacity ran almost parallel. This observation also does not quite fit in with the assumption that the indophenol reducing properties of normal urine is entirely due to the presence of ascorbic acid. If the whole of the indophenol reducing properties is attributed to the presence of ascorbic acid it is difficult to understand why it should have any bearing upon the protein intake and metabolism.

On the bases of these observations we made some experiments with a view to see if there was any relation between the indophenol reducing properties of the urine and purine metabolism. Uric acid content and the reducing capacity of the urine were determined simultaneously in a number of normal as well as pathological cases, with the following results :—

TABLE IV.

Nature of specimen.	Reducing property in terms of ascorbic acid mg. in 100 c.c.	Uric acid in grammes per litre.	REMARKS.
1. Normal (S. D.) ..	2.5	..	..
2. do. (A. C. R.) ..	4.0	..	..
3. do. (S. D.) ..	2.66	..	..
4. do. (H. M.) ..	2.90	0.32	..
5. do. (A. C. R.) ..	5.3	0.53	..
6. do. (S. D.) ..	less than 0.4	0.16	..
7. do. (P. B.) ..	3.0	0.44	..
8. do. (A. C. R.) ..	5.0	0.47	..
9. do. „ ..	3.0	0.44	..
10. do. (S. D.) ..	1.6	0.26	..
11. do. „ ..	1.4	0.22	..
12. do. „ ..	1.6	0.33	..
13. do. (P. B.) ..	2.3	0.34	..
14. do. (S. L.) ..	2.2	0.32	..
15. do. (N. P.) ..	3.1	0.42	..
16. do. (A. C. R.) ..	2.9	0.42	..
17. do. (S. D.) ..	2.0	0.16	..
18. do. (A. C. R.) {	3.7	0.33	Immediate.
	3.7	0.33	After 4 hours.
19. do. „ ..	2.9	0.32	..
20. do. (H. M.) ..	4.4	0.44	..
21. do. (A. C. R.) ..	4.5	0.45	..

TABLE IV—*contd.*

Nature of specimen.	Reducing property in terms of ascorbic acid mg. in 100 c.c.	Uric acid in grammes per litre.	REMARKS.
22. Normal (S. D.) ..	5.0	0.44	This individual is not a habitual meat or fish eater and in previous cases his urine gave low values but on inquiry it was found that he took fairly large quantities of fish on that as well as the previous day.
23. do. (A. C. R.) ..	5.0	0.53	..
24. do. „ ..	5.0	0.47	..
25. Polyarthritis ..	6.2	0.59	..
26. Gout (before atophan)	7.7	0.64	..
27. „ (after atophan)	6.2	0.48	..
28. Cured E. D.; takes plenty of red atta, meat and fruits.	14.2	0.70	..
29. do. ..	16.6	0.64	..
30. do. ..	14.2	0.50	..
31. Epidemic dropsy ..	1.0	..	..
32. do. ..	1.54	..	..
33. do. ..	1.32	..	..
34. do. ..	1.32	..	..
35. do. ..	1.10	..	..
36. do. ..	2.3	0.35	..
37. do. ..	0.9	0.14	..
38. do. ..	1.1	0.21	..
39. do. ..	0.8	..	..
40. do. ..	1.6	..	..
41. do. (convalescent).	6.0	0.54	..
42. do. ..	0.8	0.21	..

TABLE IV—*concl'd.*

Nature of specimen.	Reducing property in terms of ascorbic acid mg. in 100 c.c.	Uric acid in grammes per litre.	REMARKS.
43. Epidemic dropsy ..	0.8	0.21	..
44. do. ..	1.4	0.26	..
45. do. ..	2.0	0.33	..
46. do. ..	3.2	0.42	..
47. do. ..	0.8	0.21	..
48. do. ..	1.4	0.26	..
49. do. ..	2.0	0.33	..
50. do. ..	3.2	0.42	..
51. do. ..	2.0	0.32	..
52. do. ..	2.1	0.38	..
53. do. ..	2.1	0.33	..

These results indicate that :—

1. There is an undoubted relation between the indophenol titre and the uric acid content of the urine and that there is even a rough proportion between the two in the majority of cases.

2. The excretion of the reducing substances as also of uric acid is low in the urine of persons suffering from epidemic dropsy.

3. That the indophenol titre and the uric acid content increase considerably after the epidemic dropsy patients have been put on for some time to high protein and fruit diets and when all active symptoms of the disease have disappeared.

4. The urine of one individual (S. D.) had usually very little reducing action but on a particular day the titre was found to increase considerably and it was found that on that day and the previous night he had liberal quantities of fish.

5. In one case simultaneous determination of the indophenol titre and uric acid content were made immediately after the urine was passed and, also after 4 hours to see if there was any simultaneous change of the uric acid content with the decrease of the titre value due to keeping, but in this case both sets of values were the same.

Three individuals were put on to a diet containing a large amount of fat (ketogenic diet—240 grammes of fat in 24 hours). After three or four days, acetone bodies were found in the urine in appreciable quantities. The indophenol titres of these specimens were low, showing that a diet rich in fat does not increase the reducing properties of the urine.

We had also the opportunity of testing a few specimens of aqueous humour of the eye and found that they readily reduced indophenol.

1. 12.5 mg. per 100 c.c.

2. 6.6 mg. „ „ „

But these are glaucoma fluids from epidemic dropsy cases kindly supplied to us by Lieut.-Colonel E. O'G. Kirwan, I.M.S., Professor of Ophthalmic Surgery, Medical College, Calcutta, and may have lower reducing property than normal specimens. We were not fortunate enough to secure aqueous humour of the eye from normal individuals and therefore comparisons could not be made.

SUMMARY AND CONCLUSIONS.

1. The evidence put forward to establish the identity of the indophenol reducing substance in the urine of normal individuals and in various other tissues, with vitamin C is not conclusive.

2. The biological tests give conflicting results and are unsatisfactory.

3. The titre varies considerably according to the nature and strength of the acids used for titration.

4. Trichloroacetic acid, acetic acid and sulphuric acid are none of them efficient preservatives for urine, but 5 per cent trichloroacetic acid seems to be better than the other two in this respect.

5. With 10 per cent acetic acid consistent lower values are obtained than with trichloroacetic acid.

6. The indophenol reducing property of the urine of individuals suffering from conditions other than scurvy (viz., epidemic dropsy) and even of some normal individuals is usually found to be very low but no apparent symptoms of scurvy are manifest in these cases.

7. There is a significant relation between this reducing property and the uric acid excretion, and the results run almost parallel.

8. The indophenol titre of the urine is not increased on a ketogenic diet.

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PHARMACOLOGICAL ACTION OF CAMPHOR AND ITS DERIVATIVES.

BY

BREVET-COLONEL R. N. CHOPRA, C.I.E., K.H.P., M.A., M.D. (Cantab.),
M.R.C.P. (Lond.), I.M.S.,

CAPTAIN J. S. CHOWHAN, M.B., B.S., A.I.R.O.,

AND

N. DE, M.B.

Indigenous Drugs Inquiry, Indian Research Fund Association.

(From the Department of Pharmacology, School of Tropical Medicine, Calcutta.)

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INTRODUCTION.

CAMPHOR has been used in Chinese medicine for many centuries and has also been employed in Western therapeutics during recent years. It is a favourite remedy in almost every Indian home for a variety of ailments. The reputation of camphor as a circulatory stimulant has been widely believed, but recently there has been difference of opinion among the laboratory and clinical workers regarding its pharmacological action and therapeutic uses. Camphor has been claimed to act rapidly as a cardiac stimulant by Gorman (1920). The claim for such action in the face of slow absorption and conversion into an inert compound with glycuronic acid is difficult to explain. It has been suggested that the stimulant action may be merely a reflex action as in the case of alcohol or ether administered either by mouth or subcutaneously. The experimental work done on animals has given variable results regarding its cardiac stimulant properties. While reviewing the literature on the action of camphor on the heart of a frog, Heathcote (1923) pointed out that some observers have found it to be a cardiac depressant, others as a cardiac stimulant and still others believe that it has no action on the normal heart at all. It only stimulates the heart that has been depressed artificially by toxic drugs. By his own observations on the hearts of frogs, rabbits and cats, he showed that there is no convincing pharmacological evidence in support of the view that camphor possesses any value as a cardiac or a circulatory stimulant. Cushney declared that there is no evidence to show that camphor even in large therapeutic doses has any effect except to cause a slight stimulation and this is probably due to reflex dilatation of the skin vessels. In the frog it causes a descending paralysis. Nakagawa (1923) believed that camphor affects the origin and conduction of cardiac impulses and in large doses stimulates

the latent automatism. The heart is slowed down by camphor and its derivatives both in man and in animals. Camphor has been reported to dilate the coronary vessels, but it is not certain that this occurs with therapeutic doses. Sanders (1929) reported that camphor diminished the force of contraction in dog's heart which was regularly stimulated with electric current.

Clinically camphor has been used as a rapid and effective cardiac restorative when the heart is failing or when death is impending. Efforts have been frequently made to determine how camphor acts as a stimulant when pharmacological experiments have shown it to be inactive or even depressant. It has been suggested that probably camphor undergoes some metabolic changes in the body during absorption and a hypothetical stimulant substance is liberated. Efforts have been made by various workers to synthesize such products of camphor which would probably be formed inside the body between its absorption and excretion.

melting points, colour and structure of a new set of camphor compounds sent to us for pharmacological study :—

TABLE I.
Salts of camphor.

Salts of camphor.	Colour and shape.	Melting point.	Optical rotation.	REMARKS.
(a) <i>Water-insoluble camphors.</i>				
d-camphor ..	White crystalline	178°C. to 179°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = +55.54^\circ$	Soluble in alcohol and glycerol. Action depressant throughout; chances of emboli; slight stimulation in diseased or artificially slowed heart.
l-camphor ..	Brown crystalline	178°C. to 179°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = -53.05^\circ$	
ll-camphor ..	White crystalline	..	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = 0^\circ$	
borneol ..	White amorphous	204°C. to 205°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = -42.62^\circ$	
camphor quinone.	Deep yellow accicular.	199°C. to 200°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = -176.0^\circ$	do.
amphor ..	Deep yellow fine accicular crystals.	199°C. to 200°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = +175.7^\circ$	
amphor ..	Deep yellow amorphous creamy.	198°C. to 199°C.	$[a] \text{Hg}_{\text{gr.}}^{35^\circ} = 0^\circ$	
(b) <i>Water-soluble camphors.</i>				
odio-iso-oso camphor	Deep yellow amorphous.	..	..	Loses its colour to light yellow.
dio-iso-oso camphor	Pale yellow	..	..	Inclined to be white.
o-iso-nitroso phor (race-	Pale yellow	..	..	Like above but slightly increased stimulation and rapid absorption.

There is no record in the literature regarding the difficulty of intravenous ministration of the insoluble isomers of camphor. Camphor is so sparingly luble in water that intravenous injection of this drug in Locke's solution had be given up as such large quantities had to be injected that the blood pressure se to a considerable extent due to the amount of fluid injected and therefore scured the real effects of the drug itself.

(a) *Insoluble camphors*.—Three solvents were used, i.e., alcohol (absolute), glycerol and olive oil. Control experiments with the pure solvents in all the three cases showed varying degree of pharmacological effect. Alcohol (50 per cent), even in doses of $\frac{1}{2}$ c.c. administered intravenously to cats weighing 2 to 3 kilos, anæsthetized with chloralose, often produced cardiac embarrassment as shown by a sudden fall in the blood pressure and stoppage of its pulsation which gradually returned to normal after 3 to 5 minutes. The respiration was affected which also had an indirect effect on the circulatory system. The use of alcohol as a solvent had to be finally given up as the dilute forms of alcohol did not dissolve the camphor properly. Glycerol was next tried. It was found to be less toxic than alcohol but it was also not free from untoward effects. Half to one c.c. of glycerol administered intravenously under similar conditions as the above showed, sometimes later, a gradual fall in blood pressure which returned to normal gradually after 3 to 5 minutes. Olive oil, $\frac{1}{2}$ c.c. to 1 c.c., administered under similar conditions in about 50 per cent of animals showed a slight and slow fall of blood pressure which gradually returned to normal. In the case of glycerol and olive oil, sometimes other effects like irregularity of heart beat and embarrassment of breathing occurred, due most probably to fine emboli formation in the pulmonary blood vessels and capillaries of the brain.

Finally, olive oil was preferred as solvent for the insoluble camphors for experimental work on animals. One per cent solution of the various camphor compounds in olive oil was prepared. The injection had to be given slowly with the normal saline running continuously from the burette into the vein, otherwise the oil floated back in the burette instead of going into the vein. The rubber-tubing connecting the burette to the femoral veins had also to be milked down after each injection so as to force out any oil that may be sticking in the lumen of the tube and is washed out again by letting more saline. Without these precautions the camphorated oil would stick in the tube or float back instead of completely entering the venous system. The effects produced by different camphor compounds are tabulated in Table II :—

TABLE II.

Pharmacological action of camphor.

Camphor.	Blood pressure.	Myocardio-graph.	Isolated heart.	Depressed heart.	Intestine volume.	REMARKS.
(a) <i>Insoluble camphors</i> .						
d	Fall ..	..	..	Slight stimulation.	Slight increase.	Heart depressed with quinine, emetine, hæmorrhage, etc.
dl	Fall irregular with big dose.	..	..	Nil	Big dose fall.	
I	Fall, less marked	Loss of tone	..	Nil	Slight increase.	
Borneol	Fall, not marked	Slight loss of tone.	..	Nil	do.	

TABLE II—concl'd.

Camphor.	Blood pressure.	Myocardiograph.	Isolated heart.	Depressed heart.	Intestine volume.	REMARKS.
(b) Insoluble camphors (camphor quinone).						
d	Slight stimulation, later on fall.	Depressed but irregular.	Temporary rise (marked).	..	..	(d) Frog's heart depressed, stimulation after a latent period.
dl	Slow and irregular fall.	..	do.	..	..	
l	Fall ..	..	do. (less).	..	..	
(c) Water-soluble camphors (sodio-iso-nitroso camphor).						
d	Less fall ..	Pulse 200-160	Tone increased 1-500,000 and 1-1,000,000	Temporary rise.	..	(d) Intrahepatically 1 per cent solution stimulation slowing, later on periodicity missing beats (frog's heart).
dl	Irregular ..	Pulse 200-180	Less.	Less.	..	
l	Pulsation imperceptible.	Pulse 200-180 irregular.	Coronary flow reduced, missing beat.	Less.	..	

In Sections (a) and (b) of the table it will be seen that the blood pressure is lowered in all cases with d-, dl-, and l-camphors. The quino-compounds showed a tendency of transitory stimulation which was followed by a fall which occurred gradually and was maintained. The frog's heart was depressed, but with d-camphor there was a slight improvement after a latent period. In the hearts of the cat and the rabbit when depressed artificially by giving doses of quinine, emetine, antimony or in cases of hæmorrhages and shock, etc., 2 mg. to 3 mg. of camphor per kilo body-weight produced a slight and temporary rise particularly in case of d-camphor. l-camphor produced the same effect but to a lesser degree; dl- and borneol camphor produced practically no remarkable effect. The rise of blood pressure was slight and temporary followed by a fall which did not reach such a low level as with ordinary camphors. The heart remained irregular.

(b) *Water-soluble camphors*.—Water-soluble camphor compounds in the form of sodium iso-nitroso camphors (d-, l- and dl- isomers) were used. These compounds were readily soluble in normal saline and yielded a clear pale yellow solution. The effect on the blood pressure, myocardiograph and the isolated heart was studied. A little more detailed action was studied on the hearts of frogs. The results are tabulated in Section (c) of Table II. It will be observed that the blood pressure is reduced though the effect is rapid and temporary in nature. The heart beats were

slowed but the muscular tone was increased in the isolated heart when it was perfused with dilutions of 1 in 500,000 to 1 in 1,000,000. The coronary circulation was reduced. The rabbit's heart was stimulated with 1 in 1,000,000 dilutions of d-camphor derivative but it was soon followed by depression; slowing and later irregularity of beats followed. The l-camphor derivative produced only a mild stimulation with 1 in 50,000 to 1 in 100,000. There was a fall of blood pressure in cat when 10 mg. of d-camphor derivative was injected intravenously. The blood pressure gradually returned to normal. This effect was practically absent in l- and dl-camphor derivatives. The pulse rate was reduced from 200 to 160 beats per minute with d-camphor derivatives. The other two isomers did not produce such a marked effect. The heart volume, the auricles and ventricular beats were not effected at first but bigger doses lead to dilatation and irregularity of rhythm. With d-camphor derivatives a periodicity was commenced. There was missing of beats both in the auricle and ventricle after every 16 beats. In the case of frog the action of these camphors was much more delayed. In some cases there was a slight stimulation augmenting the contraction and the number of beats. There appeared to be a slight stimulation when 0.1 c.c. of a 1 per cent solution of d-camphor derivatives was injected intrahepatically. The stimulation was more marked though much delayed in occurrence. After about 10 minutes a periodicity was noticed and there was a block in the conduction of impulses through the bundle. These effects disappeared after washing the heart with fresh saline. The other two isomers did not produce any marked effects.

DISCUSSION AND CONCLUSIONS.

The natural camphor and its synthetic isomers should be regarded, pharmacologically, as depressant to the isolated heart as well as in intact animals. When injected intravenously into cats and rabbits, camphor produces a gradual and prolonged fall of blood pressure. There may sometimes be noticed a mild stimulant effect after a prolonged perfusion. The isolated frog's heart (Straub's method) is depressed in various concentrations, while in weak concentrations and after a latent period the heart appeared to be slightly improved in its beats. The nutrient fluid, containing weak concentrations of camphor and which has been kept in contact with the heart or other living tissue for a certain length of time, if perfused through another isolated heart, improves it slightly. This shows that camphor probably when coming in contact with a living tissue or when it has remained in the body for a certain length of time undergoes some biochemical changes and then produces diametrically opposite effects on the heart. Similar changes may possibly be taking place inside the body after the administration of camphor and may possibly explain its marked cardiac stimulant effect when used clinically. During the pharmacological experiments the metabolic and oxidation processes may not take place so rapidly with insoluble camphors and therefore the stimulant effects are not manifested. Intra-arterial injections are known to be more depressant than intravenous injections. The hypothetical products, possibly an intermediate between camphor and its excretion product in combination with glucuronic acid, were synthesized and studied by Tamura, Kihara and Ishidate (1930 to 1934). The stages of oxidation of camphor inside the body possibly are: (1) The camphoral (p-o-oxy-camphor) which was found to be slightly stimulant after an initial depression and latency. (2) The next product in order of formation is a glucoside of p- and o-oxy-camphor which

experimentally produced no stimulant effect on the heart. The other possible intermediate products of metabolism are o-oxo-camphor (o-Diketo camphene) and p-oxo-camphor (p-Diketo camphene). Of these two, while the former has no effect the latter is markedly stimulant to the perfused frog's heart. Intravenous injection of this latter product in frogs and rabbits, in doses of 0.1 c.c. of 1 per cent solution, produced immediate and marked stimulant effects. The action of camphor inside the system may thus be : (1) The original camphor and its final oxidation products are directly depressant to the isolated heart and the circulatory system. (2) The intermediate products of oxidation particularly p-oxo-camphor and o-oxo-camphor produce a marked stimulation on the heart. (3) The immediate stimulation of camphor after an injection may be merely a local reflex effect. (4) The secondary maintained and prolonged effect may be due to the slow absorption and gradual conversion of camphor into intermediate oxidation products which supply stimulation to the exhausted heart. This chemical synthesis inside the body occurs most probably inside the liver. It has been observed that if the liver is damaged or is not functioning properly, camphor does not produce its stimulant effects, but on the other hand has been reported to produce toxic effect in the individual (Sabatine, 1925). Intravenous and intrahepatic injections of camphor into frogs produced a slight stimulant effect on the heart, whilst the intra-arterial injection was always depressant in action.

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SOME INORGANIC PREPARATIONS OF THE INDIAN INDIGENOUS MEDICINE.

Part II.

BANGA BHASMA (CALCINED TIN).

BY

BREVET-COLONEL R. N. CHOPRA, C.I.E., K.H.P., M.A., M.D. (Cantab.),
M.R.C.P. (Lond.), I.M.S.,
S. GHOSH, D.SC., F.R.S.E.,

AND

ASHUTOSH DUTT, B.SC.

(From the Departments of Pharmacology and Chemistry, School of Tropical
Medicine, Calcutta.)

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TIN occurs in nature in combination with oxygen in the mineral Cassiterite or Tinstone, which is more or less a pure form of tin dioxide, SnO_2 . It is found also as tin pyrites and sometimes as a silicate, but the principal source of tin is the dioxide. Tin has been known in India from ancient times. Its vernacular name is *Banga* in Sanskrit and *Rāṅg* in Hindi and Bengali. It is said to occur in the Peninsular India and in the district of Hazaribagh in Bihar. The chief source of tin in and near India is Burma, Tenasserim and Malaya Archipelago.

Like other metals which have been used in old Hindu medicine, tin has been used in the form of a crude oxide which is prepared by a complicated process of so-called 'purification', but which really reduces it to a state of impure oxide.

The following process is generally adopted for this purpose: Metallic tin is heated in an iron pan until it is melted and the molten mass is poured into the milky juice of Arka (*Calotropis gigantea*). It is then re-melted with one-fourth of its weight of Yabaksara (impure carbonate of potash) and powdered husk of tamarind is added to it. The whole mass is stirred well with an iron-rod till it is reduced to a very fine powder. The powder is then washed with cold water and dried over a gentle fire. Another method of preparation is to heat the metal on fire in an iron pot; when molten, powdered turmeric, Jirak (*Cuminum cyminum*), Trifala, i.e., the three fruits Haritaki (*Terminalia chebula*), Bahera (*Terminalia bellerica*) and Amlaki (*Phyllanthus embelica*), powder of Aswath (*Ficus religiosa*) and tamarind barks are put in one after the other and stirred. The next powder is only put in when the one previously added is thoroughly burnt. The product thus obtained is a greyish white fine powder and is known as *Banga Bhasma* or ash of tin.

According to well-known Ayurvedic physicians, *Banga Bhasma* is used in the following diseases with different vehicles (Anupana), usually in combination with other mineral preparations like the Bhasmas (ashes) of gold, silver, zinc, iron, etc. Sometimes it is used alone. Its main indication is in inflammatory and suppurative conditions of stomach, urethra and other mucus surfaces. It is believed to be a general tonic and alterative and is often combined with *Silajatu* and *Abhra Bhasma* for this purpose. Its chief uses are in diabetes, spermatorrhœa, gonorrhœa, anæmia, asthma, gastric ulcer and in various skin diseases. The dose is from one to four grains.

We analysed a sample of *Banga Bhasma* supplied by the Kalpataru Ayurvedic Pharmacy of Calcutta. The sample was a dull-grey amorphous powder with a slightly metallic and saline taste. It was soluble in hot water to the extent of 1·12 per cent. The chemical composition found as the result of qualitative and quantitative analyses is given below :—

	Per cent.
Oxide of tin, SnO_2	82·94
Silica, SiO_2	6·38
Iron and alumina, Fe_2O_3 , Al_2O_3	2·96
Lime, CaO	1·92
Magnesia, MgO	0·69
Potash, K_2O	2·96
Soda, Na_2O	0·45
Chlorides	0·11
Moisture	0·89
Other constituents	0·70
TOTAL ..	100·00

The solubility of *Banga Bhasma* in dilute hydrochloric acid of a strength approximating that found in gastric juice was also studied. For this purpose 2·0 g. of the *Bhasma* were digested in 200 c.c. of 0·3 per cent of hydrochloric acid at a temperature of 37°C. for 24 hours. The total solubility was 7·726 per cent. The soluble portion when analysed quantitatively was found to have the following composition :—

	Parts.
Oxide of tin, SnO_2	1·060
Silica, SiO_2	0·342
Lime, CaO	2·072
Iron oxide, Fe_2O_3	0·243
Aluminium oxide, Al_2O_3	0·137
Magnesia, MgO	0·371
Potash, K_2O	2·967
Soda, Na_2O	0·424
Chlorides	0·110
TOTAL ..	7·726

TIN IN PHARMACOLOGY AND THERAPEUTICS.

Experiments have shown that when soluble salts of tin are given to animals, a small quantity is absorbed and accumulates in the tissues and tin appears in the urine. When soluble salts are given by subcutaneous injections, elimination is slow

and occurs mainly by the alimentary tract, but somewhat by the urine also. Diuresis results after administration of small doses but large doses have a deleterious effect on the kidneys and produce pathological changes in this organ. Large quantities of tin are retained in the body after administration of which 20 to 25 per cent is in the skin and 5 per cent in the liver.

It will be seen from the data given above that appreciable quantities of oxide of tin occurring in the *Bhasma* will be dissolved in the physiological acid of the gastric juice and will be absorbed into the system. Like other heavy metals small quantities of tin have a stimulant action on the central nervous system and also on hæmopoietic system and in this way have a general stimulant action and may be beneficial in such conditions as diabetes. The diuretic action is beneficial in chronic gonorrhœa and possibly traces of the metal in the urine may have some inhibitory effect on the organism responsible for this disease.

The action of tin on the heart is like that of arsenic and is probably through the vagus. It is possible that in its beneficial effects in asthma it may act by depressing the vagi in the same way as arsenical compounds like soamin do.

The use of tin in the treatment of skin diseases by the Ayurvedic physicians is rather interesting in view of the fact that there is a tendency for the metal to accumulate in the skin. It has been observed that workers in tin mines do not suffer from furuncles and based on this observation *stannoxyd* was introduced which has been successfully tried in patients suffering from furunculosis.

It may be worth while extending these trials in the treatment of such chronic and persisting diseases as eczemas, psoriasis, etc.

The nervous system is especially sensitive to this metal and large doses may produce peripheral neuritis, excitability and sclerosis of the brain or the spinal cord.

The therapeutic effects of tin compounds, however, are not so powerful as some of the compounds of other metals in use in Western medicine. Further investigations were therefore not considered necessary.

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THE HUMORAL TRANSMISSION OF THE EFFECTS OF CARDIAC VAGUS AND SYMPATHETIC STIMULATION BY DRUGS.

BY

R. K. PAL, D.Sc., M.R.C.P. (Edin.), M.Sc., M.B. (Cal.), F.R.S.E.,
Formerly Professor of Physiology, Prince of Wales Medical College, Patna.

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LOEWI (1921) showed that stimulation of the vagus produced some chemical substance which if allowed to pass on into a fresh heart produced in the second heart the same effect as if the vagus nerve of the latter had been stimulated. He also stimulated the sympathetic nerve to an isolated perfused frog's heart and could demonstrate some other chemical substance in the lumen of the ventricle, which when transferred to another heart produced in the second the effects of sympathetic stimulation. These findings were corroborated later on by other workers including Finkleman (1930) who recorded the spontaneous movements of two separate suspended strips of isolated small intestine, through the lumen of which saline was perfused from one to the other, and demonstrated thereby that stimulation of the sympathetic fibres to the first caused the relaxation of both the pieces (the second after a short latent period) and thus proved that some chemical substance formed in the first by the sympathetic stimulation was responsible for the same effect in the second. Similarly, Cannon *et al.* (1931) found that stimulation of the sympathetic nerves to the skin of cat's tail caused the liberation of a chemical substance with adrenaline-like effects, into the blood-stream. That a similar substance was liberated during life when the sympathetic nerves were stimulated, was also clearly demonstrated by them. Bain (1932) described a method of demonstrating humoral transmission of the effects of cardiac vagus stimulation in the frog and showed that when the vagus of the donor heart was stimulated for forty seconds the heart stopped in about fifteen seconds with slight slowing of the recipient heart which also came to a standstill after another fifteen minutes or so. He confirmed his finding regarding the liberation of the vagus substance on stimulation of the vagus nerve to the donor heart by allowing perfusion fluid to flow from it to an isolated stomach whose movements were also simultaneously recorded. The stomach gave a contraction of relatively large amplitude, attaining its maximum rapidly when the vagus nerve to the heart was stimulated for about 15 seconds causing stoppage of the heart; on discontinuing the vagus stimulation the stomach relaxed again.

From all these experiments there seems to be little doubt regarding the production of vagus or accelerans substance in the frog's heart caused by the stimulation of vagus and sympathetic respectively by electrical stimulation. This paper attempts to show whether there is production of any chemical substance in the heart by stimulation or inhibition of the vagus or the sympathetic by means other than electrical stimulation, e.g., certain drugs that act on these nerves.

EXPERIMENTAL.

Frogs were used for the experiment and heart to heart perfusion was done by Bain's (*loc. cit.*) method, slightly modified (*see* Diagram). The inflow cannula (*E*) to

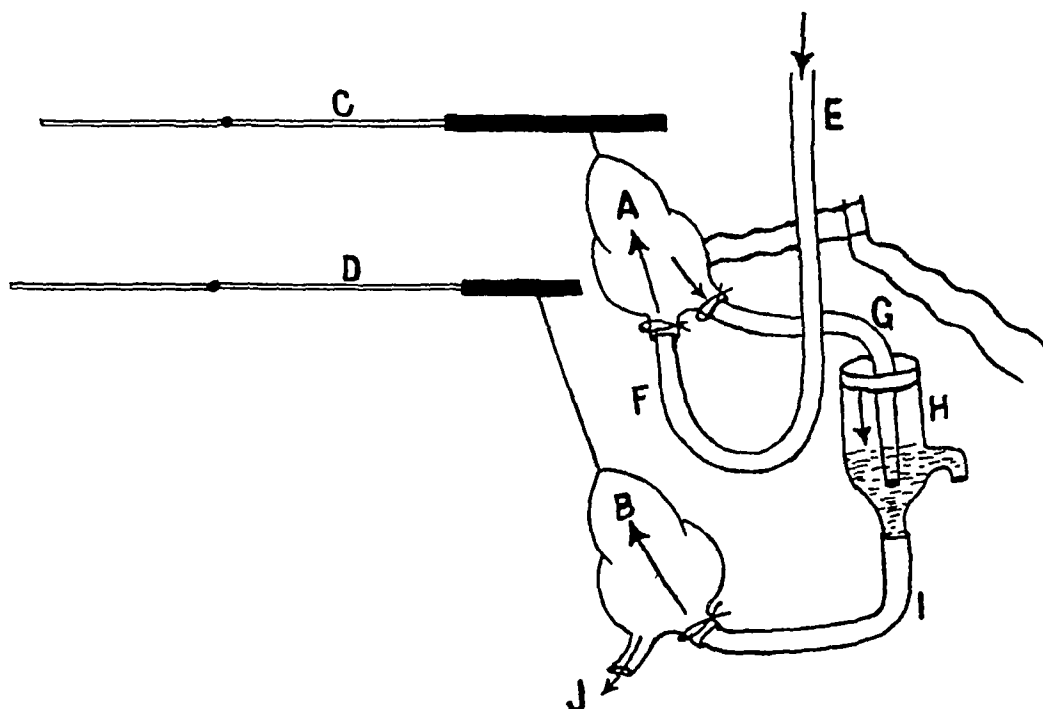


DIAGRAM OF THE APPARATUS.

- A — Donor heart.
- B — Recipient heart.
- C and D — Levers to which A and B are attached.
- E — Inflow tube to the donor heart.
- F — Cannula into inferior vena cava.
- G — Outflow tube from the donor heart.
- H — Glass cannulated tube.
- I — Inflow tube into the recipient heart.
- J — Outlet from the recipient heart through a cut into the aortic arch.

the donor heart was introduced through the inferior vena cava instead of through the right auricle. The outflow tube (*G*) from the donor heart was introduced into the first part of the arch of the aorta (with its branches ligatured) and passed into the glass cannulated tube (*H*). From the nozzle of the latter a venous cannula

connected by a very small piece of rubber-tubing (I) went into the inferior vena cava of the recipient heart. A cut in the aortic arch formed the outlet for the fluid from the second heart. The fluid used for perfusion was modified Ringer's solution for frogs (Macleod, 1930). The drugs used for the experiment were adrenaline chloride (P. D.), ergotoxin (B. W.), acetylcholine (B. D. H.), insulin (Lilly brand), pilocarpine nitrate, nicotine and atropine sulphate. The drugs were all dropped on the donor heart so as to stimulate or paralyse the sympathetic, the vagus or the ganglion as the case may be.

RESULTS.

(See Cardiographs.)

Adrenaline.—When 3 drops of adrenaline chloride (1 in 1,000) were dropped on the donor heart the rate as well as the amplitude of contraction were increased. The recipient heart which was working rather slowly with some irregularity, not only became regular but also showed much improvement in rate as well as the force of contraction (Fig. 1).

Ergotoxin.—Ten drops of ergotoxin (0.065 per cent solution) caused some slowing of the heart. The recipient heart also showed same effect with diminution in amplitude. Curiously enough, ergotoxin had not much appreciable effect on a fresh heart and the effect manifested itself when the heart was working for some time (Fig. 2).

Nicotine.—Five drops of 1 per cent solution caused slowing with much diminution in amplitude of contraction of the donor heart, which was later on followed by much improved beats (in amplitude as well as in rate). The same effect was also noticed on the recipient heart (Fig. 3).

Acetylcholine.—Fifteen minims of 5 per cent solution when dropped on the donor heart caused complete stoppage of the heart which could be revived only by a few drops of atropine (10 drops of 0.5 per cent solution) by counteracting the action of the former on the heart. The recipient heart too showed analogous effects (extreme slowing with lowering of the amplitude), which became improved again when the donor heart was acted upon by atropine (Fig. 4).

Pilocarpine.—Ten drops of 1 per cent solution after a short latent period produced slight slowing of the donor heart. The same was also noticed on the recipient heart with occasional missing of the beats (Fig. 5).

Insulin.—Five units when dropped on the heart caused slowing in the rate of contraction with some irregularity in the donor heart. The recipient heart was also slightly slowed down with diminution in amplitude, which regained its former height as the donor heart was partly shaking off the effects of insulin (Fig. 6).

Atropine.—0.5 per cent solution had very little effect when applied to a fresh heart, nor was the recipient heart affected in any way. But it had its effects markedly on a heart already slowed down under the influence of some other antagonistic drug, e.g., acetylcholine and the recipient heart was also affected similarly (Fig. 4). In the same way it can also counteract, when previously administered, the effects of the antagonistic drugs on the donor and recipient hearts, to some extent.

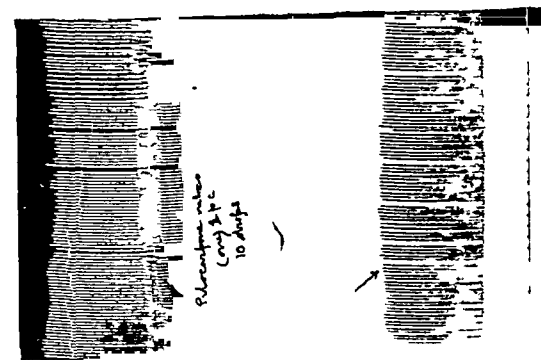
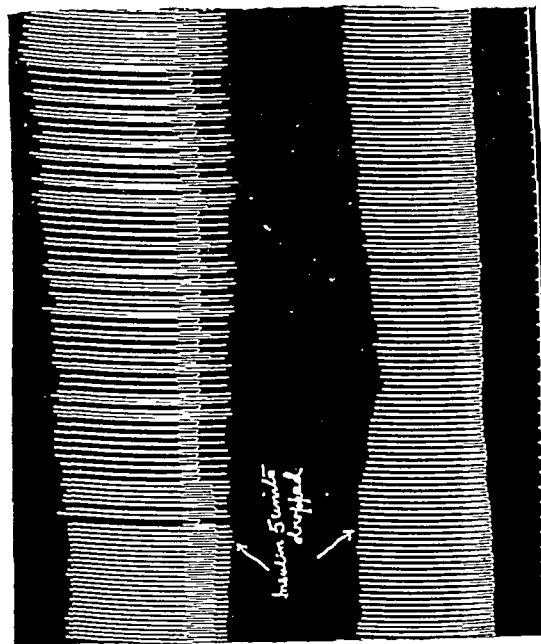
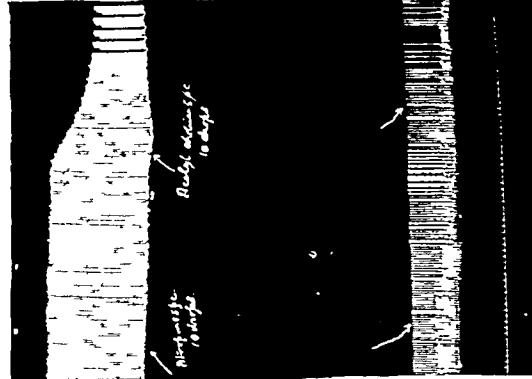
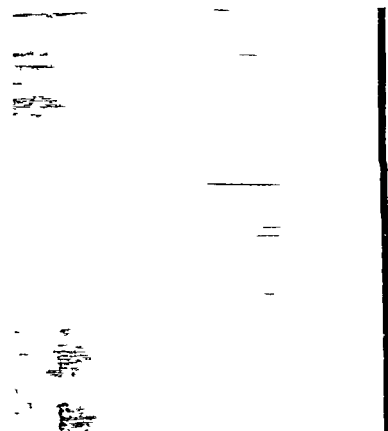
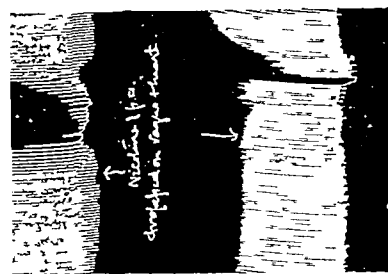
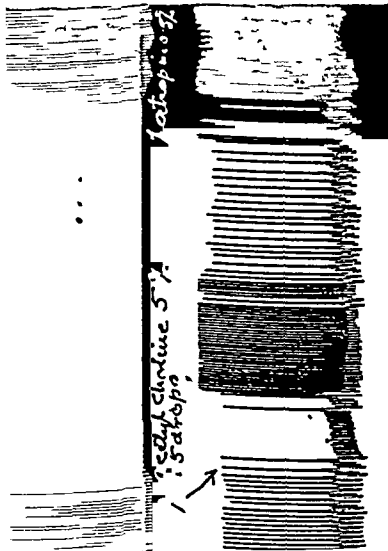


Fig. 7.

Fig. 6.

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Fig. 1.—Upper, donor heart; lower, the recipient heart, time 5 seconds. Fig. 2.—Ergotoxin, 10 drops of 0.065 per cent on the donor heart—slowing in rate and diminution in amplitude of the recipient heart corresponding to the effects on the donor heart. When the donor heart was partially recovering from the effects of the drug the recipient heart too showed improvement in amplitude. Fig. 3.—Five drops of 1 per cent solution of nicotine—extreme slowing with diminution in amplitude of the donor heart followed by improvement in amplitude of the recipient heart also. The drop of 5 per cent solution—complete stopping of the donor heart followed by acceleration and augmentation of the recipient heart.

Fig. 7 shows only some diminution in amplitude with slowing of the hearts after 10 drops of acetylcholine, on a heart previously acted upon by atropine (10 drops) even without any appreciable effect, the recipient heart too showing slowing in rate.

DISCUSSION.

From the above results it is evident that the drugs that act on the autonomic nervous system (sympathetic, para-sympathetic and ganglia) are capable of producing some chemical substance in a heart which if allowed to pass on into another heart will affect the latter similarly. Drugs that act on the sympathetic by stimulation, as adrenaline, produce something in the heart that causes acceleration and augmentation as well in the recipient heart. The reverse effects are produced by the inhibitor of the sympathetic, e.g., ergotoxin. Nicotine first stimulates and then paralyses the ganglia; this also produces something in the donor heart that has at first a slowing and then stimulating action on the recipient heart. Acetylcholine, pilocarpine, insulin (Pal and Prasad, 1934), etc., all stimulate the vagus endings and consequently produce the vagus substance which leads to effects on the second heart similar to those after stimulation of the vagus by electric current. Atropine, being the paralysing agent of the vagus terminals, has the reverse effect especially after the action of the antagonistic drugs and to minimize the action of such drugs when administered previously.

Curiously enough, the drugs that have a stimulating influence on the sympathetic or the vagus have got more marked effects than their physiological antagonists. In this respect ergotoxin and atropine, though less effective, still produce positive results under certain conditions. Some observers such as Stefan (1928), have not been able to find any positive result with atropine and ergotamine. The reason probably is that the effects of negative stimulation of the nerves do not come in as spontaneously and quickly as those of positive stimulation. So it can be said definitely that as vagus substance and 'sympathin' (Cannon *et al.*, *loc. cit.*) can be produced by stimulation of the vagus and sympathetic respectively, the drugs that stimulate the nerves can also produce allied substances which when transferred to another heart will lead to corresponding effects of vagus and sympathetic stimulation respectively. Even the antagonistic drugs such as ergotoxin and atropine that paralyse the sympathetic and vagus respectively, if allowed to act on a donor heart are capable of producing anti-vagus and anti-accelerans substances (anti-sympathin?) with typical effects of paralysis of the respective nerve terminals on the recipient heart.

As the method of heart to heart perfusion described in the text ensures a constant flow under a constant pressure, the effects on the recipient heart can be taken without any doubt as due to some chemical substances produced in the donor heart under the influence of various drugs.

CONCLUSION.

Drugs acting on the vagus and sympathetic by stimulation are capable of producing vagus and accelerans substances respectively in the heart. The antagonistic drugs that have a paralysing action on the nerve terminals can also produce anti-substances when they are allowed to act on a heart. The negative stimulation

produced by them is undoubtedly less effective for the liberation of specific chemical substances than the positive stimulation. Drugs acting on the ganglia also produce some corresponding chemical substance in the heart.

SUMMARY.

Humoral transmission of the effects of drugs acting on the autonomic nervous system has been definitely proved.

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A STUDY ON THE ACTIVATION OF TISSUE-GROWTH (IN VITRO) WITH COBRA VENOM.

BY

BREVET-COLONEL R. N. CHOPRA, C.I.E., K.H.P., M.A., M.D. (Cantab.),
M.R.C.P. (Lond.), I.M.S.,

N. N. DAS, M.Sc., M.B.,

AND

S. N. MUKHERJEE, M.Sc.

(From the Department of Pharmacology, School of Tropical Medicine
and Hygiene, Calcutta.)

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INTRODUCTION.

THE cobra venom is said to have remarkable therapeutic properties in many pathological conditions, and in small doses it is believed to have marked stimulating effects on certain tissues. In large doses it is also known to affect certain tissues and its powerful effect on the nervous tissue in particular is well known. The peculiarity of the action of this venom is that in very minute doses it has stimulant effects on the nerve cells and is useful in therapeutics, but in larger doses the action is positively harmful, causing paralysis and death of these cells. Although all these facts have been known, the way in which this action is brought about and the nature of such action have still remained obscure. The present work was undertaken to study the effect of this venom on the growth of tissues cultivated *in vitro* and thereby to throw some light, if possible, on its mode of action.

EXPERIMENTAL PROCEDURE.

The tissues were obtained from chick's embryo ten days old. Freshly laid eggs were carefully incubated at 40°C. for ten days, after which the embryo was removed with aseptic precautions. The tissue from the choroid coat of the eye was always used in these experiments since this tissue has been found to grow well under artificial conditions, and being of a membranous nature the growth can be easily pursued. Very small pieces of this tissue (of the size $\frac{1}{2}$ mm. sq.) were taken with edges clearly cut. The clear-cut edges are absolutely essential in these experiments since with rough edges it is difficult to follow the progress of the growth.

Before actually proceeding with the experiments, embryo extract was prepared by cutting the remainder of embryo in small fragments and by stirring these with a

ramrod in Pannet's solution. The clear supernatant liquid obtained after centrifugalization gave the necessary extract. The chick plasma was also obtained from blood drawn from the wing vein of a cock. As a precaution against clotting, the whole operation of drawing blood and separating the plasma was carried out with a cold paraffined syringe and cold test-tube. The plasma was also stored in the cold.

After these preliminary operations, hanging drop preparations on coverslips with a drop of plasma and the tissue extract were made in which the choroidal tissue was explanted. These were incubated at 37°C. and observations were made at intervals of 24 hours up to 60 hours and the results noted.

In experiments with cobra venom explants were made as before, the only difference being that a drop of the venom solution of a given concentration prepared in Pannet's solution was mixed with it in addition. Controls in this case were kept by adding a drop of Pannet's solution in the explants instead of a solution of venom.

RESULTS AND DISCUSSION.

The results obtained have been given in the Table. A perusal of this table will show that with cobra venom in dilution from 1 in 1,000 to 1 in 20,000, the growth was definitely inhibited, while in dilutions of 1 in 50,000 and upwards the growth appeared to be stimulated. In all the series of more than 150 experiments for each dilution uniform results were obtained.

TABLE.

Showing the relative growth of tissues.

COBRA VENOM DILUTIONS.

Experiment number.	1 in 100.			1 in 1,000.			1 in 10,000.			1 in 20,000.			1 in 50,000.		
	Number of control.	Number of experiment.	Results.	Number of control.	Number of experiment.	Results.	Number of control.	Number of experiment.	Results.	Number of control.	Number of experiment.	Results.	Number of control.	Number of experiment.	Results.
1	5	5	—	5	6	—	7	9	—	6	5	±	6	5	+
2	10	12	—	10	8	—	6	7	±	6	9	±	8	12	±
3	8	10	—	12	11	—	3	4	—	10	6	±	8	10	+
4	9	12	—	9	12	*	9	6	—	7	8	—	9	12	+
5	10	16	—	10	8	—	8	5	±	9	12	±	10	8	+
6	10	12	*	15	12	—	12	8	—	7	3	—	8	6	±
7	12	15	—	6	8	—	6	7	—	5	8	—	10	8	+
8	9	6	—	9	12	—	5	5	±	9	12	±	12	12	+
9	8	10	—	5	7	±	9	8	*	7	10	+	14	10	+
10	5	7	..	4	6	—	7	4	—	6	5	±	9	8	+

— less growth in comparison to control.

± doubtful increase of growth.

+ growth increased compared with the control.

* These experiments were vitiated due to contamination and are therefore inconclusive.

It will be observed from the Graph that in dilutions of 1 in 100 or in 1 in 1,000 the growth is totally inhibited, while in dilutions of 1 in 10,000 and 1 in 20,000 there is slight growth although it is much less than the control. But in dilutions of 1 in 50,000 or in 1 in 80,000 the growth is much greater than that of the control. The curve for the growth for the dilution of 1 in 80,000, however, runs below that for 1 in 50,000 at which dilution, therefore, the growth seems to be at its maximum. The Curve showing growth with dilution is given.

It is well known that for the growth of a tissue two factors are mainly responsible; the first being the support on which the tissue grows and the second being the presence of certain substances in the medium which promote growth. Carrel (1912) found that the best support for growth is the fibrin network of the blood plasma. Carrel and Baker (1926) explained the stimulating action of the embryo extract as due to the proteolytic products formed by the hydrolysis of proteins by the cell enzymes. He also showed that the higher proteolytic products such as proteoses, etc., have a stimulating action upon the tissue-growth, whereas peptone and other smaller split products, although appear to furnish some nutrient material, do not, however, cause rapid proliferation characteristic of proteoses; on the other hand, they often prove toxic for tissue cells, especially when present in larger amounts. These authors also studied the action of the hydrolytic products of commercial fibrin and produced further evidence in support of their views.

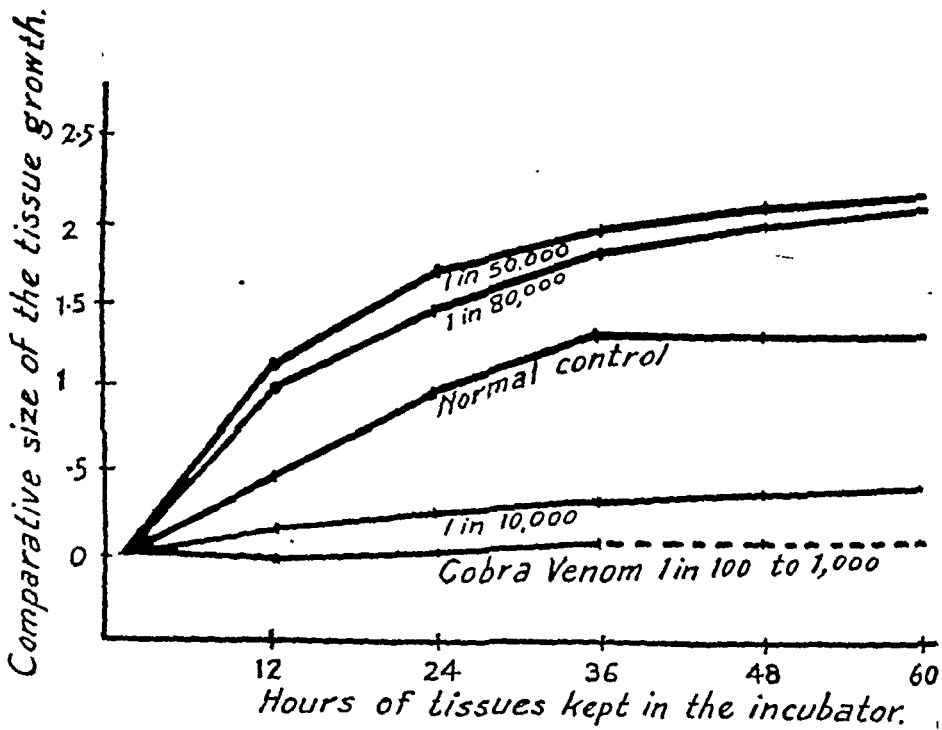
Cobra venom, since it affects the growth of tissues, must influence one or other of the two factors responsible for tissue-growth. It may have influence either on the fibrin network which acts as a support or it may act by changing the medium only.

As early as 1902 Flexner and Noguchi observed that the cobra venom could digest fibrin which forms the support for the growth of tissues in the present case. Since then different authors established the fibrinolytic properties of venom in general and cobra venom in particular. The recent work of Chopra and Roy (to be shortly published) has definitely established the same point and has given a comparative study of the more common snake venoms so far as their proteolytic actions are concerned. Martin (1905) also found fibrin ferments in various venoms and he was of opinion that these ferments were probably responsible for thrombosis.

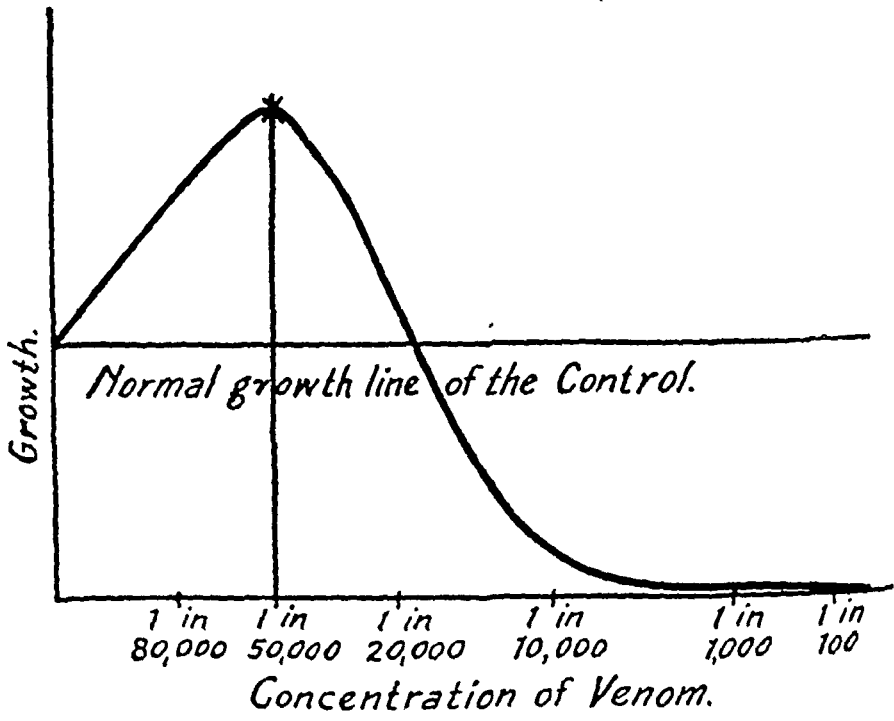
In the light of these contributions it is not difficult to form an idea as to how venom affects the growth of tissues. In the first place it stimulates the formation of thrombin (Houssay and Sordelli, 1920) and thereby the production of fibrin. Secondly, the enzymes present in the venom also have a hydrolytic action on the fibrin. Thus the two antagonistic actions proceed side by side. In course of some experiments carried out in this laboratory on the coagulation of blood it was observed that the cobra venom promoted coagulation of blood in small concentrations only, whereas in larger concentrations the blood did not coagulate at all. This evidently shows that in smaller concentrations the venom promotes the formation of thrombin more than the destruction of fibrin, while in larger concentrations the fibrinolytic action is more pronounced and for this reason the blood cannot coagulate.

The importance of fibrin in the present experiments on the cultivation of tissues consists in this that it forms the support on which the tissue grows. At low concentrations wherein the formation of fibrin is believed to be promoted as evidenced by increased coagulation of blood, the growth of the fibroblasts is evidently at its

GRAPH.



CURVE.



maximum. On the other hand at concentrations where coagulation experiments show a maximum fibrinolytic action of the venom the growth of tissues is inhibited. This parallelism of the tissue-growth with the formation and destruction of fibrin seems to be very suggestive, inasmuch as the fibrin network appears to be a strong influencing factor for the growth of tissues, in presence of cobra venom.

Over and above this there is another factor which seems to be equally important. From the work of Carrel and Baker (*loc. cit.*) it is evident that higher proteolytic products stimulate the growth of tissues, while lower products may prove toxic and consequently inhibit growth. It is not, however, unlikely that higher split products like proteoses are formed only when the concentration of venom is small, whereas with larger concentrations of venom other subsequent products are possible. In the light of this in lower concentrations a stimulation of growth might be expected due to presence of proteoses, whereas in higher concentrations an inhibition is possible. The fibrin would thus appear to be of great importance for the growth of tissues. The support which is formed of fibrin network has therefore a strong influence on the cultivation of tissues.

Last but not least there are also several physico-chemical alterations that are brought about by the mere introduction of the venom or by its proteolytic reactions. These physico-chemical factors no doubt act as auxiliary factors in course of the growth of tissues, but the direction in which they act is not yet very clear.

SUMMARY AND CONCLUSIONS.

1. Cobra venom in higher dilutions stimulates the growth of tissues *in vitro*, while at lower dilutions it inhibits growth.

2. This promotion and inhibition of growth have been explained as due to the different types of enzyme action on fibrin. At higher dilutions the formation of fibrin is promoted by fibrin ferments for which it is likely that the growth is also promoted. At lower dilutions the fibrinolytic action of the venom is more pronounced for which the support on which the tissue grows is likely to be affected, and in consequence the growth is inhibited.

3. The proteolytic products of fibrins are also responsible for growth. At higher dilutions the growth is promoted due to the formation of proteoses, while at lower dilutions smaller split products probably prove toxic to the cells.

4. The physico-chemical changes brought about by the introduction of venom and by its proteolytic activity must also have an effect, but the direction in which such changes act are not known.

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THE ACTION OF RATTLESNAKE AND MOCASSIN VENOMS AS COMPARED WITH INDIAN VIPER VENOMS.

BY

COLONEL J. TAYLOR, D.S.O., M.D., D.P.H., V.H.S., I.M.S.,

AND

CAPTAIN S. M. K. MALLICK, M.B., M.R.C.P., M.R.C.S., I.M.S.

(*An Inquiry under the Indian Research Fund Association.*)

(*From the Central Research Institute, Kasauli.*)

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THE receipt of a small supply of the venoms of the American vipers *Crotalus adamanteus* (rattlesnake) and *Ancistrodon piscivorus* (mocassin) has enabled a comparison to be made between the action of these venoms and that of the Indian vipers *V. russellii* (daboia) and *Echis carinata* (phoorsa), the results of the study of which have already been reported (Taylor *et al.*, 1935a, b, and c).

CROTALUS ADAMANTEUS VENOM.

Neurotoxic action.

The rapidly fatal action of the intravenous injection of the venom in standard pigeons of 300 g. weight was taken as an indication of the neurotoxic content of the venom. A series of pigeons were given intravenous injections of a solution of the dried venom in a strength of 1 mg. per c.c.

Pigeon No. 1. Dose 0.1 mg. Symptoms of shock, vomiting after 27 minutes, recovery.

„ No. 2. Dose 0.15 mg. Similar symptoms, recovered.

„ No. 3. Dose 2 mg. Immediate symptoms of shock, pigeon collapsed and lying on side, slow respiration, slight convulsions, died in 16 minutes.

„ No. 4. Dose 0.25 mg. Similar symptoms, died in 17 minutes.

The minimum lethal dose can be taken to be about 0.2 mg. which is 20 times that for *daboia* venom (0.01 mg.) and four times that for *echis* venom (0.05 mg.)

At post-mortem examination the two pigeons that died showed intense congestion of the vessels of the splanchnic area and engorgement of the abdominal viscera. The left side of the heart was empty and the right side distended. No clots were noted in the vessels and the blood did not coagulate on standing overnight in paraffined tubes. The cause of death would appear to have been an extreme fall in blood pressure following paralysis of the splanchnic capillaries, the action being the same as that described by Chopra and Chowhan (1934) in the case of *daboia* venom poisoning.

Neutralization of neurotoxin by daboia antivenene.

In previous work with viper venoms we have found that all those so far examined, including some Indian, European and African vipers, possess specific neurotoxic properties and that their *neurotoxins* were not neutralized by the heterologous antivenenes which were available for testing. Neutralization of the immediate lethal effect of the rattlesnake venom by the antivenene prepared at Kasauli, of which 1 c.c. unconcentrated serum neutralizes 1 mg. of *daboia* venom and 1 c.c. concentrated neutralizes 4 mg., was tested.

The following mixtures were injected intravenously in standard pigeons:—

0.4 mg. rattlesnake venom (= 2 m.l.d.) + 1 c.c. unconcentrated antivenene—died in 12 minutes.

0.4 mg. rattlesnake venom (= 2 m.l.d.) + 1 c.c. concentrated antivenene—died in 17 minutes.

In a proportion equivalent to ten times its titre against *daboia* venom the serum had no neutralizing action on rattlesnake venom.

An unconcentrated *echis* antivenene was also available for testing but this was only of low titre, 1 c.c. neutralizing 0.5 mg. of the *echis* venom. An intravenous injection of a mixture of 2 c.c. of the antivenene and 0.4 mg. (= 2 m.l.d.) of rattlesnake venom caused the death of 2 standard pigeons in 5 minutes. This antivenene did not neutralize the neurotoxic action of the venom in proportion of two and a half times its titre. These results are in line with our previous experience of the specificity of this action of viper venoms.

Hæmorrhagin content.

The *hæmorrhagin* content of the venom was determined by intracutaneous injection on the skin of the abdomen of white rabbits. 0.1 c.c. of a series of dilutions of the venom was injected and the production of hæmorrhage noted at the end of one hour.

Venom dilutions	..	1/10,000	1/50,000	1/100,000	1/500,000
Results	..	+	—	—	—

The minimum skin hæmorrhagic dose was 0.01 mg. which is the same as has been found for the venoms of *Echis carinata* and *V. berus* and one-tenth of that found for *daboia* venom. *Crotalus adamanteus* venom thus comes into the group of venoms of high *hæmorrhagin* and low *neurotoxin* content.

Neutralization of the hæmorrhagin by daboia antivenene.

The hæmorrhagins of all viper venoms which we have previously examined were found to be neutralized by antivenenes prepared against different viper venoms (Taylor and Mallick, 1935a). Neutralization of hæmorrhagin of rattlesnake venom with our antivenene was tested by intradermal injection of mixtures of the venom and the serum. The following doses were given :—

Antivenene + Venom solution.	Dose.	Number of skin doses.	Result.
1 c.c. + 1 c.c. (= 0.1 mg.)	0.2 c.c.	1	—
1 c.c. + 1 c.c. (= 0.2 mg.)	0.2 c.c.	2	+
.. 1 c.c. (= 0.1 mg.)	0.1 c.c.	1	+

The hæmorrhagin of the venom was neutralized in accordance with the titre of the serum and in relation to the relative hæmorrhagin content of the *daboia* and rattlesnake venoms.

The neutralization of the hæmorrhagic action of the venom of *daboia* antivenene was further tested *in vivo*. It had been observed during experiments with *echis* venom (Taylor and Mallick, 1935b) which in *neurotoxin* and *hæmorrhagin* content is very similar to rattlesnake venom, that the results of doses which are not rapidly fatal were mainly of hæmorrhagic nature and could be largely prevented by the use of heterologous viper antivenene. This was tested in rabbits, one rabbit being given 3.0 mg. of the rattlesnake venom subcutaneously on the thigh and another the same dose along with 7.5 c.c. of concentrated antivenene, which is the calculated dose for neutralization of its hæmorrhagin. After 24 hours the control rabbit showed extensive hæmorrhagic swelling over the whole thigh spreading into the scrotum and on to the lower part of the abdomen. The skin of the scrotum sloughed on the following day and the animal was chloroformed. On examination a gelatinous œdema with hæmorrhage was found covering both thighs, the scrotum and lower part of the abdomen, but no definite changes were found in the internal organs or other parts of the body. The rabbit which received antivenene presented a marked contrast in appearance. A slight degree of scrotal swelling without discoloration was noted at the end of 24 hours and when killed 48 hours after the injection only a limited area of œdema with slight blood staining was present. The dose given to the control animal had caused a serious injury which would probably have had fatal results, while in the serum-treated rabbit the injury was minimal and would easily have been recovered from.

Coagulant action.

Employing the technique previously used for testing the coagulant action of venoms (Taylor *et al.*, 1935c) in which venom solutions were added to fresh sheep's

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blood the following rates of coagulation were observed with different final dilutions of the venom in blood :—

Venom dilutions ..	1/10,000	1/50,000	1/100,000	1/200,000	1/500,000	1/1,000,000	Saline control.
Coagulation time in mins. and secs ..	0-42	3-45	9-10	13-40	17-35	20-20	29-15

The venom has an active coagulant action in the lower dilutions but is less active than *daboia* or *echis* venoms in the higher dilutions.

Neutralization of coagulant action by heterologous antivenene.

It had been observed (Taylor *et al.*, 1935*a* and *c*) that although the *hæmorrhagin* of viper venoms which had been examined was neutralized by a heterologous antivenene, neutralization of coagulant action was not obtained. A short series of tests of neutralization was carried with dilutions of a mixture of 1 c.c. of unconcentrated *daboia* antivenene and 1 c.c. of a solution of rattlesnake venom containing 1 mg. The final dilutions of venom + serum mixture in sheep's blood and the coagulation times were as follows :—

Venom and serum dilutions. ..	..	1/20,000	1/25,000	1/50,000	Saline control.	Venom control.
Coagulation time in mins. and secs. ..	..	1-50	2-0	2-15	19-6	1-45

The coagulant action of the venom was not neutralized by the heterologous serum and the observation is in accordance with previous findings which indicate the specificity of the coagulant action of viper venoms. Our observations on this and other viper venoms would suggest that the coagulant action of viper venoms is dependent on a specific cytolytic action resulting in the release of thrombokinase.

In the course of experiments carried out in monkeys in which death occurred after a period of delay the blood was found to be incoagulable. As the coagulant action of the rattlesnake venom is similar to that of *daboia* and *echis* venoms the explanation of this finding which we have given (Taylor *et al.*, 1935*c*) for the later venoms, namely, slow defibrination with deposition of fibrin in the vessel walls, will also apply to this venom.

ANCISTRODON PISCIVOROUS VENOM.

Neurotoxic action.

Tests were carried out on pigeons on the same lines as with rattlesnake venom.

Pigeon No. 1.	Dose 0.025 mg.	Survived.
„ No. 2.	Dose 0.05 mg.	Survived.
„ No. 3.	Dose 0.10 mg.	Symptoms of shock similar to those observed with rattlesnake venom. Died in 14 minutes.
„ No. 4.	Dose 0.15 mg.	Similar symptoms. Died in 18 minutes.

The minimal lethal dose is about 0.1 mg. which is one-half of that of the rattlesnake venom, ten times that of *daboia* venom and twice that of *echis* venom. The post-mortem appearances were similar to what have been found in the case of pigeons dying rapidly from the intravenous injection of other viper venoms.

Neutralization of neurotoxin by daboia antivenene.

Tests were carried out in pigeons by intravenous injection of mixtures of mocassin venom solution and *daboia* antivenene both in concentrated and unconcentrated form with the following results:—

Venom dose + Antivenene.	Result.
0.2 mg. (= 2 m.l.d.) + 1 c.c. unconcentrated.	Died in 5 minutes.
0.2 mg. (= 2 m.l.d.) + 1 c.c. concentrated.	Died in 5 minutes.
0.1 mg. (= 1 m.l.d.)	Died in 20 minutes.

The concentrated antivenene would neutralize at least 4 mg. *daboia* venom by the method of test but does not neutralize a twentieth part of the amount of mocassin venom.

In a similar test with *echis* antivenene 2 c.c. of the serum failed to neutralize 0.2 mg. (=2 m.l.d.) of the mocassin venom, the pigeon dying in 3 minutes. The serum in this case was used in a proportion representing 5 times its homologous titre. The neurotoxin is shown by these tests to be specifically different from that of *daboia* or *echis*.

Hæmorrhagin content and its neutralization.

The skin hæmorrhagic dose was ascertained by the intradermal test to be 0.01 mg. The hæmorrhagin content of the venom is thus similar to that of the rattlesnake and *echis* and ten times that of *daboia* venoms.

The hæmorrhagin was neutralized to titre by *daboia* antivenene in exactly the same degree as the hæmorrhagin of rattlesnake venom. It is thus homologous with the hæmorrhagin of the other viper venoms which we have examined.

Action of mocassin venom on blood coagulation.

The coagulant action of this venom on sheep's blood in paraffined tubes was tested by the method employed for rattlesnake venom with the following results:—

Venom dilutions ..	1/10,000	1/50,000	1/100,000	1/500,000	1/1,000,000	Saline control.
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Coagulation time ..	No clot at 18 hrs.	28 mins.
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Firm clot at 18 hrs.

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A repetition of the test gave similar results and the venom is shown to have a definite anti-coagulant action which we have not observed in any other viper venom we have tested.

An attempt was made to determine whether mocassin venom would neutralize the coagulant action of rattlesnake venom and a series of mixtures of the two venoms were made and added to fresh sheep's blood. With proportions up to twenty parts of mocassin venom to one part of rattlesnake venom no appreciable prolongation of the coagulation time was observed.

The action of mocassin venom solution was also tested on solutions of fibrinogen to which thrombin had been added. In a series of 5 tubes a mixture consisting of 5 drops fibrinogen and 2 drops of thrombin solution was placed and the following additions were made to successive tubes, the results being noted after incubation at 37°C. overnight :—

No. 1.	1 drop of 1/1,000 mocassin venom	..	..	no clot.
No. 2.	2 drops of 1/1,000 „ „	..	..	no clot.
No. 3.	1 drop of 1/1,000 <i>daboia</i> „	..	..	clot.
No. 4.	2 drops of horse serum	..	..	clot.
No. 5.	2 drops of saline solution	..	..	clot.

The anti-thrombic action of mocassin venom is further shown by these findings.

The results of these tests of rattlesnake and mocassin venoms in comparison with the venoms of the *daboia* and *echis* are summarized in the following Table :—

TABLE.

Venom.	Lethal dose for pigeons, mg.	Neurotoxin.	Skin hæmorrhagin dose, mg.	Nature of hæmorrhagin.	Coagulant action.
<i>V. russellii</i> ..	0·01	Specific.	0·10	Homologous for all four venoms.	Specific.
<i>E. carinata</i> ..	0·05	„	0·01		„
<i>C. adamanteus</i> ..	0·20	„	0·01		„
<i>A. piscivorus</i> ..	0·10	„	0·01		Anti-coagulant.

The specificity of the neurotoxic action of the rattlesnake and mocassin venoms has only been determined in relation to the Indian snake venoms which themselves differ specifically in their action. The relationship between the neurotoxins of rattlesnake and mocassin venoms was not determined as no homologous anti-sera were available.

SUMMARY.

The results of a study of the neurotoxic, hæmorrhagic and blood-coagulant actions of the venoms of the American vipers *Crotalus adamanteus* and *Ancistrodon*

piscivorous in comparison with the action of the venoms of the Indian vipers *V. russellii* and *E. carinata* is reported.

The two American viper venoms have a high *hæmorrhagin* content similar to that of *echis* venom and ten times that of *V. russellii* venom. The *hæmorrhagin* of all four venoms is of the same nature and is neutralized to titre by *daboia* antivenene. The *neurotoxin* content of the *rattlesnake* venom is one-twentieth of that of *daboia* venom and one-quarter of that of *echis* venom. The *neurotoxin* content of *mocassin* venom is one-tenth of that of *daboia* venom and half of that of *echis* venom. The *neurotoxins* of the *rattlesnake* and *mocassin* venoms differ specifically from those of *daboia* and *echis* and are not neutralized by large excess of anti-sera prepared against these venoms.

The *rattlesnake* venom has definite coagulant action on blood but is much less active in high dilutions than *daboia* or *echis* venom. Its coagulant action is not neutralized by *daboia* antivenene. *Mocassin* venom has an anti-coagulant action on blood.

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STUDIES ON INDIAN SNAKE VENOMS.

Part II.

COBRA VENOM: ITS CHEMICAL COMPOSITION, PROTEIN FRACTIONS AND THEIR PHYSIOLOGICAL ACTIONS.

BY

S. N. GANGULY, PH.D. (Lond.),

AND

M. T. MALKANA, M.SC.

Inquiry under the Indian Research Fund Association.

(From the Central Research Institute, Kasauli.)

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A STUDY of the chemical composition and location of the toxic principles of cobra venom was made on the same lines as the present authors had employed in the case of daboia venom (Ganguly and Malkana, 1936).

CHEMICAL ANALYSIS OF THE INDIAN COBRA VENOM.

The preliminary examination showed the presence of the following elements : C, H, N, S, O and P. Certain inorganic substances and yellow pigments to which the colour of the dried venom is due were also present.

The venom gave the usual reactions for protein and the identification of the proteins was undertaken. The proteins present were found to be (1) coagulable proteids consisting of albumin and globulin, (2) non-coagulable proteids consisting of primary and secondary proteoses only. As phosphorus has been found in this venom detailed examination was made for phospho-proteins, but none were present.

Ether extraction of the dried venom resulted in removal of a dark brown viscid substance which was identified as a mixture of lecithin and cholesterol.

In addition to the free lecithin which was extractable with ether, a part of it was found to be present in combination with the venom proteins in the form of lecitho-proteins.

The total nitrogen content of the venom as estimated by a modified micro-Kjeldahl method, the details of which have been described in the previous paper (*loc. cit.*), was found in successive tests to be on the average 15.06 per cent. Out of this total, however, the nitrogen content of the protein fraction as obtained by precipitation with trichloroacetic acid or sodium-tungstate was ascertained to be 14.01 per cent, representing 87.56 per cent ($N \times 6.25$) of total protein. The non-protein nitrogen as determined from the supernatant fluid left after precipitation of the proteins from the venom solution was estimated to be 1.05 per cent.

DETERMINATION OF THE PROTEIN FRACTIONS OF THE VENOM.

The more complex composition of this venom as compared with that of the *daboia*, necessitated the employment of methods different in principle from those employed previously, for the fractionation of the constituent proteins. The methods devised for the present purpose are described below :—

Estimation of proteoses.—One per cent venom solution in saline (15 c.c.) was heated to 95°C. on a water-bath for about twenty minutes, till the coagulable proteids were completely coagulated. The coagulum was filtered off and washed and the clear filtrate, representing the primary and the secondary proteoses together with non-protein nitrogenous substances, was treated with an equal volume of fifty per cent trichloroacetic acid to precipitate the proteoses from the rest of the contaminating substances. The precipitate was filtered, washed with 25 per cent trichloroacetic acid and then dissolved in the smallest amount of caustic soda necessary to bring it into solution. It was then neutralized with acetic acid and the volume brought back to the original (15 c.c.) by the addition of distilled water.

The nitrogen content of 2 c.c. portions of the solution thus obtained, representing the amount of primary and secondary proteoses present in 20 mg. of the dried venom, was estimated in successive tests. It was found to contain an average of 4.5 per cent of nitrogen. This is equivalent to 28.12 per cent ($N \times 6.25$) of the two proteoses in the dried venom.

Secondary protease.—The remaining solution containing the two proteoses was saturated with sodium chloride, and left in the refrigerator overnight. The following morning the precipitated primary protease which settled down was centrifuged off and the supernatant fluid containing the secondary protease alone was separated and 2 c.c. portions of this were taken separately for determination of the nitrogen content. An average of three readings showed that it contained 2.69 per cent of nitrogen in relation to the dried venom. This represents 16.81 per cent ($N \times 6.25$) of the secondary protease in the dried venom.

Primary protease.—The amount of primary protease present was calculated by subtracting the amount of secondary as determined in the previous experiment from the amount of the total proteoses present in the venom. It was thus found to be 11.31 per cent in relation to the dried venom.

Estimation of the coagulable proteids.—A fresh sample of the venom solution (5 c.c. of 1 in 100) in saline was heated as described above, for about 20 minutes at

95°C. The coagulable proteids representing the globulin and albumin were coagulated, and the coagulum was filtered and washed to remove any of the adhering non-coagulable proteids and other substances present. The nitrogen content of the coagulum as a whole was estimated in the usual way and was found to be 9.6 per cent in relation to the dried venom, which corresponds to 60.0 per cent ($N \times 6.25$) of the total coagulable proteids.

Estimation of albumin.—The globulin and the primary proteose fractions of the venom proteins were precipitated by full saturation of a 1 per cent solution with sodium chloride. The solution was allowed to stand overnight and subsequently the supernatant fluid was separated off either by centrifuging or filtration through a coarse filter-paper. Five c.c. of the clear solution thus obtained was heated at 93°C. for twenty to twenty-five minutes, in order to coagulate completely the rest of the coagulable proteids (the albumin) present in it. The coagulum was filtered, washed with distilled water and its nitrogen content was determined. It was found to contain 6.35 per cent of nitrogen in relation to the dried venom. This represents 39.69 per cent of albumin.

Estimation of globulin.—By subtracting the amount of albumin from the total coagulable proteids present in the venom the amount of globulin present was estimated to be 20.31 per cent.

As a further check, however, the nitrogen content of the proteins of the venom precipitable by full saturation with sodium chloride (representing a mixture of globulin and primary proteose) was determined and found to be 4.98 per cent, which represents 31.12 ($N \times 6.25$) per cent of the mixture. Out of this total, the amount of primary proteose which was previously determined to be 11.31 per cent was subtracted, the remainder of 19.8 per cent was, therefore, the amount of globulin present. The figure thus arrived at is slightly lower than the one previously obtained, but since the latter process is more complicated than the former one, the former figure was taken to be more correct.

The composition of the venom was thus found to be :—

				Per cent.
Protein (total)	..	..	..	87.56
Globulin ..	..	..	..	20.31
Albumin ..	..	..	..	39.69
Primary proteose	..	..	..	11.31
Secondary proteose	..	..	..	16.81

along with phospho-lipins (lecithin), cholesterol, some inorganic substances and colouring matter.

LOCATION OF THE ACTIVE PRINCIPLE IN THE VENOM FRACTIONS.

The tests of the physiological action of the fractions of the venom were carried out in regard to neurotoxin as indicated by the lethal action in pigeons on intramuscular injection. This is a routine test in use at the Institute and the sample of the venom used had an m.l.d. of 0.4 mg. for a pigeon of 300 g. weight by intramuscular injection. The dose of the venom fractions used is expressed as the equivalent of the original dried venom.

The fraction of the venom as obtained by full saturation with ammonium sulphate, representing the total proteins, was found to contain the full toxicity as judged by the lethal effect in pigeons, the m.l.d. of the protein obtained in this way being the same as the equivalent amount of the dried venom.

The fraction separated by full saturation with sodium chloride or 50 per cent saturation with ammonium sulphate, representing the mixture of the total globulin and primary proteose, was found to have no effect, pigeons surviving a dose up to the equivalent of ten times the m.l.d.

In order to ascertain whether the toxicity could be ascribed to the albumin or the secondary proteose or to both, the latter fraction was separated from the former by heating a 1 per cent solution of the venom (previously freed from the globulin and the primary proteose by full saturation with sodium chloride) to a temperature of six minutes. The albumin fraction was completely coagulated leaving proteose alone in solution. The m.l.d. of this protein fraction calculated the proportion to the original dried venom which it represented showed retained the whole toxicity of the venom. This finding shows conclusively that the albumin fraction is not the toxic one.

As mentioned here that, as in the case of daboia venom, the toxicity of cobra venom is associated with the secondary proteose, but unlike daboia the toxic fraction is to a great extent thermostabile. While heating to 75°C. for six minutes completely destroys the toxicity of the daboia venom, the toxicity of the cobra venom after similar treatment is left unimpaired.

TABLE.

Showing neurotoxic action of the protein fraction of cobra venom.

Fractions.	Mean percentage by weight in relation to dried venom.	Intramuscular dose in mg. (equivalent of dried venom) injected in pigeons.	Result.	REMARKS.
1. Total protein ..	87.56	0.4	Died	Full toxicity.
2. Globulin	20.31	Not tested.	—	—
3. Albumin	39.69	0.4, 0.6, 0.8, 1.0.	Survived.	Non-toxic.
4. Globulin and primary proteose.	31.12	4.0	„	„
5. Secondary proteose ..	16.81	0.4	Died.	Full toxicity.
6. Primary proteose ..	11.31	Not tested.	—	—

EFFECT OF HYDROLYSIS ON THE VENOM PROTEOSE.

Having ascertained that the toxicity of the venom resided entirely with the secondary proteose, further experiments were done to see whether the proteose itself or some other agent present in it, either in adsorption or some form of loose chemical combination, was responsible for its physiological action. It was proposed, therefore, to hydrolyse the secondary proteose fraction of the venom to the corresponding inactive amino-acids and then to make a study of the effect of injection of the resulting hydrolysis product in pigeons.

Since a rapid hydrolysis of the venom proteose by the usual methods of refluxing with acid or alkali involved the risk of detoxicating the proteose in the process, due either to the effect of heat or the presence of acids or alkalis, a slow process of hydrolysis by the proteolytic digestion with trypsin was employed. This process involved no risk of damaging any foreign substances in the venom proteose of non-protein origin—the action of trypsin being a selective one, namely, the hydrolysis of proteins to their higher degradation products.

Trypsin was selected as the digestive enzyme for this particular substance because, although its optimum of reaction is reached at pH 8·5, it was found to act quite satisfactorily in digesting albumins and other proteins in neutral media. Moreover, its proteolytic action was found to be much more pronounced than any of the other enzymes such as pepsin or papain under similar circumstances.

The results of control experiments done on the proteolytic action of these three enzymes on a solution of egg albumin (20 c.c.) are shown below :—

Enzyme.	Amount (in c.c.) of 0·0192 N caustic soda required for the formol titration after an incubation period of 24 hours.
1. Papain ..	27·8
2. Trypsin ..	53·6
3. Pepsin ..	25·6

Hydrolysis of the venom proteose.—A 1 per cent solution of the secondary proteose of the venom (10 c.c.), measured in relation to the weight of the dried venom, was mixed with trypsin solution (1 c.c.) and to the mixture was added normal saline (9 c.c.) to make a concentration of the proteose in the reaction mixture equivalent to 5 mg. of the dried venom per c.c. The reaction mixture was then incubated at 37°C. for a couple of months to ensure complete hydrolysis. The completeness of the hydrolysis was indicated by the reaction mixture giving a negative biuret test for the proteoses and also by the failure of an equal volume of 50 per cent trichloroacetic acid to cause precipitation of any of the unchanged proteose present.

The amount of proteose hydrolysed in the reaction mixture was also estimated quantitatively by determining the amount of nitrogen transformed into the amino-acid form by the proteolytic hydrolysis of the venom proteose. The estimation was done according to Sørensen's (1907) formol titration method which consisted

in fixing the free amino-group with formalin and then titrating the amount of resulting acidity against a standard alkali at the beginning and the end of the process. The difference between the two readings represents the amount of excess of acidity caused by the setting free of the carboxyl group of the resulting amino-acid and is taken to be a measure of the latter. The results of formol titration of 5 c.c. of the reaction mixture (representing an equivalent of 25 mg. of the dried venom) are as given below :—

Amount (in c.c.) of 0.0194 N caustic soda required in time <i>t</i> .		Difference.
<i>t</i> = 0 hours	<i>t</i> = 2 months	
0 > 7.95 7.95	0 > 10.5 10.5	2.55 c.c.

This represents 0.686 mg. or 2.7 per cent of nitrogen in relation to the dried venom. As has been previously stated, the nitrogen content of the dried venom in the secondary proteose form is on the average 2.69 per cent. Thus the secondary proteose was quantitatively hydrolysed in the process of tryptic digestion to the simple amino-acids.

EFFECT OF INJECTION IN PIGEONS OF THE HYDROLYSATE.

The resulting hydrolysis product in doses equivalent in mg. of the dried venom were injected intramuscularly in pigeons. It was observed that the pigeons survived even a dose of 6 mg., which is equivalent to 15 m.l.d's. This clearly indicates that the toxicity of the venom is due entirely to the secondary proteose and is lost when the proteose molecule is broken down to the simpler amino-acids.

SUMMARY AND CONCLUSION.

1. The chemical analysis of cobra venom shows that it contains C, H, N, S, O and P.
2. The dried venom contains 87.56 per cent protein, lecithin and cholesterol. The lecithin is present both in the free state as extractable with ether and in combination as lecitho-proteids.
3. The protein fractions are : globulin 20.31 per cent, albumin 39.69 per cent, primary proteose 11.31 per cent and secondary proteose 16.81 per cent.
4. The toxicity of the venom is entirely due to the secondary proteose and is lost on hydrolysing the proteose to the corresponding amino-acids by tryptic digestion.

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STUDIES ON INDIAN SNAKE VENOMS.

Part III.

ENZYMES IN DABOIA AND COBRA VENOMS.

BY

S. N. GANGULY, PH.D. (Lond.).

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(From the Central Research Institute, Kasauli.)

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THE results of an investigation into the chemical composition and location of the active principles of daboia and cobra venoms have been given in previous papers (Ganguly and Malkana, 1936*a* and *b*). The present paper deals with the nature of the enzymes present in these venoms. Little detailed study has previously been made on the enzymes of snake venoms. Dunn (1934) has demonstrated certain enzymes in the venom of *Crotalus adamanteus* and, amongst others, Potick (1930) has shown evidence of reduction of the phospholipoid contents of the plasma possibly due to the action of snake venoms. Houssay (1930) has also described the presence of enzymes.

The presence of phosphodiastase was also noticed by Rousseau (1934) in cobra venom.

IDENTIFICATION OF THE ENZYMES.

I. Proteolytic enzymes.

The presence of these enzymes, as indicated by their power of splitting up proteins into degradation products such as amino-acids, was investigated in the case of both cobra and daboia venoms. The substrates used for this work were (a) gelatin, (b) crystallized egg albumin, (c) casein and (d) fibrin.

Action on gelatin and egg albumin.—In studying the action of venom enzymes on these two proteins, solutions of suitable strength (approximately 2 per cent) of these substances were made in distilled water. Measured volumes (40 c.c.) of each of the solutions were mixed separately with measured volumes (4 c.c.) of 1 per cent of each of these venom solutions. Experiments showed that this amount of venom solution, representing 40 mg. of dried venom, was most convenient for the purpose; a lesser amount caused a very faint hydrolysis which was difficult to measure, whereas the use of too much venom was always avoided owing to paucity of materials.

Each of the reaction mixtures was then allowed to stand in an incubator at 37°C., and the amount of amino-nitrogen formed during the course of proteolytic

hydrolysis was measured immediately after the addition of the venom and at intervals of 24 and 72 hours, samples of 10 c.c. of each of the reaction mixtures being removed for this purpose. Controls were carried out on the same substrates with trypsin, pepsin and papain.

The tests were carried out by (1) Sørensen's formol titration method and (2) by precipitation with trichloroacetic acid.

(1) *Sørensen's formol titration method.*—The ordinary procedure of arresting the alkalinity of amino-groups of the amino-acids with commercial formalin and subsequently titrating with standard alkali (0.0192 N caustic soda) the acidity of the carboxyl groups was employed, and the differences between the initial reading and the subsequent readings were taken as indicating the amount of amino-acid formed in course of that particular period of reaction.

(2) *Precipitation with trichloroacetic acid.*—This consisted in precipitating the undigested proteins of the reaction mixture with 20 per cent trichloroacetic acid according to the method adopted by Dunn (*loc. cit.*) and determining subsequently the amount of non-protein nitrogen in the filtrate. All nitrogen estimations were done by a modified micro-Kjeldahl method as described in a previous communication (Ganguly and Malkana, 1936a).

The results of a series of tests on gelatin and crystallized egg albumin are given in Table I. It will be seen that both cobra and daboia venoms show a digestive action on gelatin and egg albumin more or less comparable with that of pepsin or papain but less active than that of trypsin. The cobra venom showed an action somewhat more marked than that of daboia venom.

TABLE I.

Showing proteolytic action of daboia and cobra venoms on egg albumin and gelatin as compared with that of known enzymes.

Protein.	Proteolytic agent.	AMOUNT (IN C.C.) OF 0.0192 N CAUSTIC SODA REQUIRED FOR FORMOL TITRATION PER 100 C.C. OF THE REACTION MIXTURE IN TIME= t .			Amino-nitrogen in mg. per 100 c.c. of the reaction mixture after digestion for 72 hours, determined by trichloroacetic acid method.
		$t=0$ hours.	$t=24$ hours.	$t=72$ hours.	
Gelatin	1. Trypsin ..	89.5	324.5	389.5	80.65
	2. Pepsin ..	97.0	164.0	197.4	26.95
	3. Papain ..	97.0	105.5	132.0	9.40
	4. Cobra venom ..	95.5	107.5	124.0	7.25
	5. Daboia venom ..	95.0	104.0	112.5	4.20
Egg albumin.	1. Trypsin ..	106.0	268.0	290.5	47.2
	2. Pepsin ..	119.5	129.0	134.5	4.2
	3. Papain ..	119.5	139.4	149.5	8.4
	4. Cobra venom ..	117.6	134.5	143.5	9.6
	5. Daboia venom ..	117.0	121.5	129.5	3.4

The presence of proteolytic enzymes in both of these venoms is demonstrated.

Action on casein.—A 2 per cent solution of casein was made in an approximately decinormal solution of caustic soda. A measured volume of the casein solution (40 c.c.) thus made was mixed separately with 4 c.c. each of daboia and cobra venom solutions (1 per cent) and incubated at 37°C. for a known length of time. A control experiment using trypsin as the digestive enzyme was made simultaneously under similar conditions. The course of the proteolytic digestion was measured in exactly the same way as in the case of gelatin and egg albumin with the slight modification that the formol titration of the reaction mixture was made against 0.02 N sulphuric acid, and the reduction in alkalinity during the period of incubation was determined and taken as the measure of the amount of amino-nitrogen liberated during the process. The results obtained are given in Table II:—

TABLE II.

Showing proteolytic action of venoms on casein as compared with the action of trypsin on the protein.

Protein.	Proteolytic agent.	AMOUNT (IN C.C.) OF 0.02 N SULPHURIC ACID REQUIRED FOR FORMOL TITRATION PER 100 C.C. OF THE REACTION MIXTURE IN TIME = t .		Amount of amino-nitrogen in mg. formed per 100 c.c. after 72 hours (as determined by the trichloroacetic acid method).
		$t=0$ hours.	$t=72$ hours.	
Casein	1. Trypsin ..	418.0	335.0	23.24
	2. Cobra venom ..	166.25	132.75	9.38
	3. Daboia venom	162.50	135.50	7.56

The results indicate that both of the venoms show action on casein in an alkaline medium. The action of cobra venom is in this case again more pronounced than that of the daboia venom.

Action on fibrin.—Fibrin prepared from sheep's blood was washed thoroughly with saline solution to remove completely the adhering red blood cells. It was then kept immersed for 24 hours in 0.5 per cent slightly ammoniacal carmine solution in water. The resulting carmin-fibrin was taken out, washed thoroughly to free it from the superfluous dye and was preserved in glycerine for further use.

Small amounts of carmin-fibrin were placed in sugar tubes, to which were added known amounts of venom solutions. The tubes were then incubated at 40°C. for known periods and the effect was observed. Controls of carmin-fibrin in saline and pepsin were also incubated under similar conditions. By the proteolytic action of the enzymes fibrin is digested with the consequent coloration of the supernatant fluid from the liberation of the dye. Absence of colour in the supernatant fluid indicated a negative reaction, the colour of the resulting fluid being

directly proportional to the enzyme activity. The following are the results of a series of tests :—

Proteolytic agent.		Results.	Incubation period.
1. Pepsin	(2 c.c. of 1 in 100)	++++	6 hours.
2. Cobra venom (	do.)	++++	„
3. Daboia venom (	do.)	+++	„
4. Saline control (	2 c.c.)	-----	„

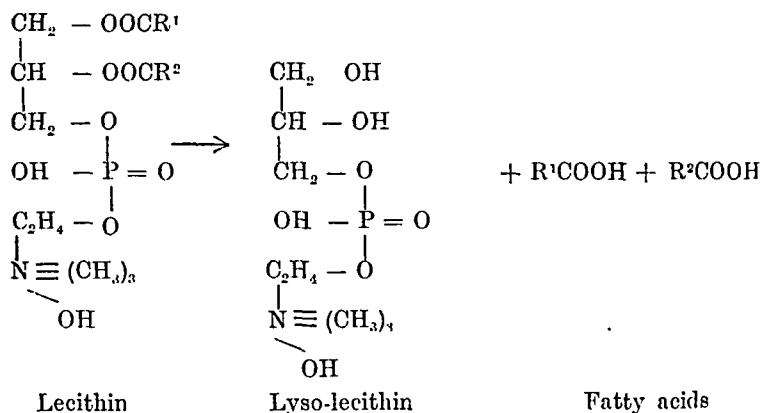
A repeat of this test on another sample using less venom after incubation for about 4 hours gave the following results :—

1. Pepsin	(2 c.c. of 1 in 1,000)	++++
2. Cobra venom (	do.)	++
3. Daboia venom (	do.)	+++
4. Saline control (	2 c.c.)	-----

The results would show the ability of these venoms to digest fibrin and confirm the findings on other substrates.

II. Lecithinase.

This enzyme possesses the specific property of hydrolysing lecithin into the corresponding lyso-lecithin, liberating in the process the constituent unsaturated fatty acids of the lecithin molecule according to the following scheme (Cameron, 1933) :—



The action of lecithinase on the lecithin molecule is thus quite different from the action of the usual hydrolytic substances on it, which result in the formation of glycerol, phosphoric acid, choline and fatty acids.

This enzyme activity was, therefore, demonstrated by determining the amount of excess of standard alkali necessary to neutralize the acid set free from the lecithin molecule during the course of the reaction.

The substrate employed for this test consisted of a lecithin emulsion in water made according to the following procedure :—

Commercial lecithin (1.5 g.) was dissolved in 2 c.c. to 3 c.c. of methyl alcohol. To the solution 100 c.c. of water was gradually added with continuous stirring, to make a uniform emulsion. The alcohol was then driven off by heating the emulsion

for a couple of hours on a water-bath, after which more water was gradually added to it with stirring to make the volume 100 c.c. again. It was then preserved with a little toluene for further use.

Measured volumes of lecithin emulsion (59 c.c.) were mixed separately with 1 c.c. of 1 per cent daboia and cobra venom solutions, and the reaction mixtures were then incubated at 37°C. Immediately after the addition of the venoms and at intervals of 4 and 24 hours, 10 c.c. samples from each of the reaction mixtures were pipetted out, mixed with 30 c.c. of absolute alcohol and titrated separately against 0.0188 N caustic soda solution. The results of titration are given below :—

Venom.	THE AMOUNT (IN C.C.) OF ALKALI REQUIRED AFTER A REACTION PERIOD = t .		
	$t = 0$ hours.	$t = 4$ hours.	$t = 24$ hours.
Cobra venom ..	7.5	8.25	8.55
Daboia venom ..	7.6	7.80	7.95

A repeat of this experiment, using stronger doses of venoms in the proportion of 2 c.c. of 1 per cent venom solutions per 25 c.c. of lecithin emulsion, gave the following results :—

Venom.	AMOUNT (IN C.C.) OF ALKALI REQUIRED AFTER A REACTION PERIOD = t .	
	$t = 0$ hours.	$t = 24$ hours.
Cobra venom ..	7.70	8.95
Daboia venom ..	7.85	8.80

These results indicate that both venoms contain a lecithin splitting enzyme, its action being of a lesser degree in the case of daboia venom.

III. Rennetic enzyme.

The power of curdling milk by converting casein into an insoluble para-casein was tested.

Whole milk (30 c.c.) was mixed with 1 c.c. of one per cent calcium chloride solution and portions of 1.5 c.c. of this calcified milk were mixed in sugar tubes with 0.5 c.c. of cobra and daboia venom solutions of different strengths. These were then incubated at 40°C., and the time in minutes and seconds required to cause a curdling of the milk was recorded. With daboia venom there was no clotting, while the results obtained with the cobra venom are given below :—

Venom dilution ..	1/100	1/250	1/500	1/750	1/1,000
Coagulation time in mins. and secs. ..	0-55	1-23	5-15	6-20	14

The above figures are the averages of several readings. There was no firm clotting in any case but simply a curdling of the milk. The results as shown above indicate that, while the cobra venom possesses a marked rennetic activity, no such action is evinced by the daboia venom.

Action of homologous antivenenes on the enzymes present in the venoms, in vitro.

In carrying out the tests on the effect of antivenene on the action of the venom enzymes, Kasauli concentrated antivenene (of a titre 1 c.c. of 1 in 4 dilution neutralizing 1 mg. of daboia and 0.5 mg. of cobra venom in standard pigeons) was used.

(a) *Effect on the proteolytic action of the venoms.*—In testing the action of antivenene on the proteolytic enzymes present in the venoms 0.5 c.c. portions of a bivalent cobra and daboia antivenene raised from horses were placed in different sugar tubes with varying proportions of the venoms dissolved in 0.5 c.c. of saline. To each of these tubes a little carmin-fibrin was added and the proteolytic action of these mixtures on fibrin was examined according to the method previously described. Control experiments with the venom solutions, saline and antivenene were made simultaneously under similar conditions. The antivenene was found to have no neutralizing action on the proteolytic actions of the venoms, but on the contrary the mixtures of venom-antivenene had a much more pronounced action than the venom alone. Moreover, it was observed that the antivenene itself had a marked proteolytic effect on fibrin.

Attempts were also made to neutralize the proteolytic action of daboia venom by means of unconcentrated antivenene raised in goats against daboia venom protease. In this case as well it was observed that this antivenene also had proteolytic activity though to a lesser extent than that of antivenene raised from horses. The results of these experiments are given below in Table III :—

TABLE III.

Showing the result of tests of neutralization of venoms by antivenene.

	Proteolytic action.	RESULTS.
1. Cobra venom alone ..	++	..
2. Daboia venom alone ..	++	..
3. Cobra venom neutralized with bivalent antivenene.	+++	No neutralization.
4. Daboia venom neutralized with bivalent antivenene.	+++	do.
5. Daboia venom neutralized with anti-daboia goat serum.	++	do.
6. Bivalent antivenene alone (from horse).	+++	Proteolytic.
7. Goat's serum	++	do.
8. Saline (control)	--	..

Effect on rennetic enzyme.—Neutralization experiments with concentrated antivenene were carried out on similar lines with regard to the rennetic enzymes present in the cobra venom. Varying amounts of venom in 0·5 c.c. of distilled water were mixed with different proportions of antivenene (by volume). The volumes of each of the mixtures were then made up to 1 c.c. by the addition of more water. Half c.c. portions of these neutralized venom samples were mixed separately with 1·5 c.c. portions of calcified milk in different sugar tubes and the time required for curdling of the milk was noticed. All the tubes were kept at a constant temperature of 40°C. The results obtained are given in Table IV :—

TABLE IV.

Showing the results of tests of neutralization of the rennetic activity of cobra venom by antivenene.

Amount of venom in mg.	Amount of antivenene in c.c.	Curdling time. Mins. Secs.
2·5	0·5	No curdling.
5·0	0·5	do.
5·0	0·25	do.
5·0	0·15	21-45
5·0	0·125	5-40
5·0	0·10	3-35
5·0	0·05	2-30

The above results show that the rennetic activity of cobra venom can be neutralized by the corresponding antivenene. One c.c. of antivenene was thus found to be capable of neutralizing the rennetic action of 20 mg. of the venom.

SUMMARY.

1. Both cobra and daboia venoms contain proteolytic enzymes capable of digesting (a) gelatin, (b) crystallized egg albumin, (c) casein and (d) fibrin.
2. Both these venoms contain a lecithin splitting enzyme, the action being of a lesser degree in the case of daboia venom.

3. A rennetic enzyme has been observed to be present in cobra venom but not in daboia venom.

4. The proteolytic action of the venoms cannot be neutralized with antivenene.

5. The rennetic action of cobra venom is neutralizable by antivenene. One c.c. of Kasauli bivalent antivenene (of a titre 1 c.c. of 1 in 4 dilution neutralizing 0.5 mg. of cobra venom in standard pigeons) neutralizes the rennetic activity of 20 mg. of cobra venom.

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BIONOMICS OF *P. ARGENTIPES*.

Part I.

THE DURATION OF LIFE IN NATURE.

BY

R. O. A. SMITH, D.T.M., I.M.D.,

S. NUKERJEE, D.SC.,

K. C. HALDER, L.M.P.,

AND

JEMADAR CHIRANJI LAL, I.M.D.

(From the School of Tropical Medicine, Calcutta.)

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THE duration of life in nature of the sandfly *Phlebotomus argentipes* was studied in connection with its rôle as the vector of *Leishmania donovani*.

For the transmission of *L. donovani* by the bite of the sandfly, it is necessary that the sandfly should live for at least six days after an infecting blood meal. By the sixth day, or by the time the sandfly is ready for its third feed—since these midges feed every third day—flagellates are usually found to have invaded the pharyngeal and buccal cavities.

That the age and maturity of a flagellate may also influence its infectivity is a point which, though not decided yet, should not be neglected.

Transmission by the bite of the sandfly has been obtained on four occasions in hamsters.

Adler and Theodor (1927) are inclined to think that flagellates of *L. tropica* developing in *P. papatasi* had to be over seven days old before they became infective. On the other hand, Shortt, Sinton and Swaminath (1935) were able to produce an oriental sore in a monkey with flagellates from a *P. sergenti* five days after it was infected. With flagellates of *L. donovani* from *P. argentipes* Shortt, Barraud and Craighead (1927) produced an infection in a mouse by the intraperitoneal

inoculation of flagellates from three sandflies, which had infecting feeds from seven to twelve days previously. No positive results were obtained by the injection of flagellates from flies at the first and second feeds.

The duration of life of these midges under laboratory conditions has been very kindly worked out for us by Dr. K. C. K. E. Raja, whose report is added in the Appendix. No record was kept of the mortality of the flies after the first and second feeds, but it would not be incorrect to estimate that very nearly 50 per cent of flies die about the time of the first oviposition, i.e., the 3rd or 4th day, and 50 per cent of the remainder about the time of the second oviposition. The conception of the life-history of these midges under natural conditions was based on the knowledge of their life in the laboratory, combined with observations on their breeding and feeding habits and general distribution in nature. It was assumed, therefore, that owing to their delicate structure, reluctance to fly any distance when disturbed, tendency to remain in dark and sheltered corners of cattle sheds and living rooms, and to breed in close proximity to such situations, these midges did not voluntarily fly far from their breeding grounds, and that a given population would feed and shelter in that site closest to their breeding grounds which afforded such requirements.

In three preliminary experiments done in 1933, of 105 wild flies marked by the dusting of very fine powdered fluorescein or eosine on them, and released in the same cattle shed where they were collected, one fly was re-captured on the 5th day after release. The poor results were attributed to improper marking methods, and as the sandfly season was rapidly terminating, experiments were suspended till the next year.

In 1934 other methods of marking were also tried, viz., the removal under ether of one middle leg of each fly, or the puncturing of one wing membrane with a fine needle.

In the first series of experiments which were done before the advent of the monsoon, a total of 667 *P. argentipes*, both wild and laboratory-bred, were marked and released on four different occasions. Collections were made at irregular intervals, but none before the 3rd day after release. Of 1,049 flies captured for examination, nine marked flies were found. Of these, 1 was found on the 3rd day, 2 on the 4th day, 4 on the 7th day and 2 on the 15th day after release. The numbers recovered were very small and quite contrary to our expectations.

To obtain some idea, therefore, of what was occurring in nature it was decided to liberate marked flies in given situations and observe their prevalence by making daily catches, beginning from the day after release.

Marked flies were released in seven different situations which included cattle sheds and living rooms, selected because sandflies were naturally present in all of them but one, which will be referred to later.

Marking was done either by the dusting of powdered fluorescein on them, or the puncture under light etherization of one wing membrane. Except in two instances the flies used were laboratory-bred ones, which were marked and released the same day that they emerged from their pupal cases.

To mark the flies with fluorescein a small quantity of the finely powdered dye was placed in a test-tube with the flies; by inverting the tube a few times a fine coating of the powder was laid on the flies. A few from each batch seemed to be

adversely affected by this procedure—they were not as active as they should be—these were excluded from the numbers released. Flies marked like this were fed under laboratory conditions, and it was noted that there was no appreciable difference in the numbers which fed or in their mortality during the subsequent three days, compared with unmarked flies. Some of these flies were tested for the presence of the dye after the first oviposition. Of 31 flies so tested 30 gave the reaction. The use of the powdered dye had this advantage, that by its use we were able to determine one of the natural enemies of sandflies.

To puncture the wing membrane of the flies, they were lightly etherized, and a fine hole made about the middle of the wing with a sharp needle. Judged by the feeding experiments in the laboratory this procedure had no adverse effect on the flies. This method of marking though it was not practised would have been more useful if it was necessary to re-mark captured flies, by making a second puncture on the same wing or marking the unpunctured one.

The results obtained in these experiments are shown in Table I as well as the situations where the flies were released, method of marking used and the day of collection. Excluding experiment No. 14 where regular catches were not made—the collectors being refused admission because one of the cows had recently calved—on 17 consecutive occasions when marked flies were released in a shed and collections made regularly, in one instance only was a fairly large number of flies recovered, 13·3 per cent of those liberated. This was the first occasion when flies were released in a dwelling house and only 30 flies were used. In the other 16 experiments the recovery rate varied from 0 out of 95 to 8·7 per cent of 80 flies released. The total recovery rate for all experiments together was 3·6 per cent.

The number of marked flies recovered was always small and the different marking methods made no appreciable difference in the recovery rate.

Of the marked flies recovered 79 were caught on the 1st day after release, 18 on the 2nd, 9 on the 3rd, 7 on the 4th, 2 on the 5th and 3 on the 6th day. After the 6th day no marked flies were found though collections were continued for several days on many occasions.

Earlier in the year, before the break of the monsoon as previously mentioned, marked flies were recovered up to the 15th day after release. The experiment was therefore repeated to observe what numbers were recoverable if no collection was done for about ten days after the liberation of marked flies. Of 428 flies released not a single one was recovered in collections made on the 9th, 12th, 16th and 19th day. Two hundred and twenty-eight of these flies were marked by wing puncture and 200 with fluorescein (*see* Table Ia).

It might here be mentioned that for this work the same three men were employed who were especially trained in the collection of these midges for periods varying from 4 to 10 years. The field work was supervised by one of the first two authors (S. M. or R. O. A. S.) once or twice a week.

It is hardly conceivable that but 3 to 4 per cent of flies present in any given situation are collected after a careful search with a torch. That a fairly large proportion of flies are missed by our methods is evident when the figures in Table II are considered.

In Table II will be found the totals of the daily collection of flies from certain sheds where the experiments were carried out. The flies have been classified according to the state of the blood meal present. Since these midges do not feed again till they have completed the digestion of the previous blood and oviposited, and as this process takes approximately three days, a rough idea of the age of the fly could be obtained. Those with a full blood meal, half or more digested meal, and fully gravid without the visible presence of blood, corresponding in age to one day, two days and three days' old flies respectively.

The proportion in which these flies were found was 56, 15 and 29 per cent respectively.

A possible explanation for these findings is that the recently fed ones were in the majority as they were collected before they found suitable hiding places to remain undisturbed, while the meal was being digested. Those of the middle category were smallest as they were already in hiding in cracks and holes in the wall from where they had no reason to move. The gravid ones were found in larger numbers probably because they were on the move again as their time for oviposition was approaching.

On the assumption, therefore, that only such flies as were in hiding were missed, it will be seen that we were recovering about 56 per cent of the flies present in a given place.

To determine whether any of the flies migrated from situations where they were released, in experiments Nos. 2 and 4, Table I, collections were made from the cattle shed where they were released as well as the dwelling house in the vicinity. This shed and house was surrounded by a thick belt of vegetation and had no other houses within a radius of 50 yards. Five hundred flies were released in this shed on two occasions and only one fly and that too a male was recovered from the dwelling house.

The observation that the proportion of gravid flies collected from any site is in keeping with the numbers of the younger flies, the fact that breeding grounds were often found close to or inside the cattle sheds and living rooms, and also that any positive evidence of migration was not forthcoming, led us to infer that the previous assumption regarding the movement of these flies was correct, and that they did not migrate to any extent. The question of their fate, therefore, naturally arose. If they were not present where they were released and not found in the surrounding houses, they must either be in hiding in places unknown to us, or they were being destroyed.

The natural enemies of these midges were therefore examined. In the stomach and gut contents of lizards it was possible to detect the typical wings of *P. argentipes*; also in examining some spiders captured in these situations, reactions suspicious of fluorescein were obtained. To verify this observation about 1,152 flies were marked and released in a shed from which only the spiders were captured for examination. Of 20 spiders examined 6 gave the test for fluorescein, showing that they were responsible for the destruction of a good number of these midges. The spiders most active in this direction were predaceous ones, and belonged to two genera. One species of the Genus *heteropoda*, and the other belonged to the family of the jumping spiders, the *Attidæ*. The web-spinning species are rather a protection to these

midges, as they take shelter on or under the webs, without getting entangled in the meshes.

These experiments were all done with laboratory-bred flies, and one experiment was therefore done with wild flies, to observe if any different results would be obtained. In experiment No. 2 (see Table Ia) 100 *P. argentipes*, which were collected mostly in one shed, were released in the same shed after they were marked with fluorescein. Among them were a few *P. papatasii*. No count was made of the latter, but it was estimated that there were not more than 10 or 12. On making daily collections from this shed only two marked flies were recovered, and both were *P. papatasii*.

In view of this result, in the last experiment done this year, *P. papatasii* were used, to determine their disposal as compared with *P. argentipes*. From four houses, three situated fairly close together and the fourth a little apart, all the *P. papatasii* and *P. argentipes* were collected, marked with fluorescein and liberated in the isolated house. Sixty-three *P. papatasii* and 21 *P. argentipes* were released, and 15 *P. papatasii* and 1 *P. argentipes* were recaptured. No marked flies were found in the other three houses which were also searched each day. In this experiment, therefore, nearly 24 per cent of *P. papatasii* were recovered. This is a high proportion of recoveries compared with 4·7 per cent of *P. argentipes* in this or 3·6, the average for the previous 18 experiments; or even 13·3 per cent, the best recovery rate in any single experiment with *P. argentipes*.

P. papatasii are far more active in their habits compared with *P. argentipes*, and it is probable that the larger proportion of recoveries was due to the fact that they were better able to elude their natural enemies. Dr. Raja's note on this finding is appended.

In the Graph overleaf are shown the numbers of flies collected daily from two sheds, as well as the irregular collections from three other sheds. It will be seen that the natural destruction prevailing coupled with the daily collection of flies, caused no marked diminution in the numbers present. That the introduction of a large number of marked flies made no appreciable difference in the numbers collected the next day is also evident.

Experiments Nos. 10 and 12 shown in Table I were conducted in a dwelling house where sandflies were very scarce, about one or two at most being found each week. The release of 75 and 100 flies on two occasions resulted in the recapture of 3 marked ones. The total number of flies collected on 16 occasions was 14. Breeding grounds were located round this house in two instances.

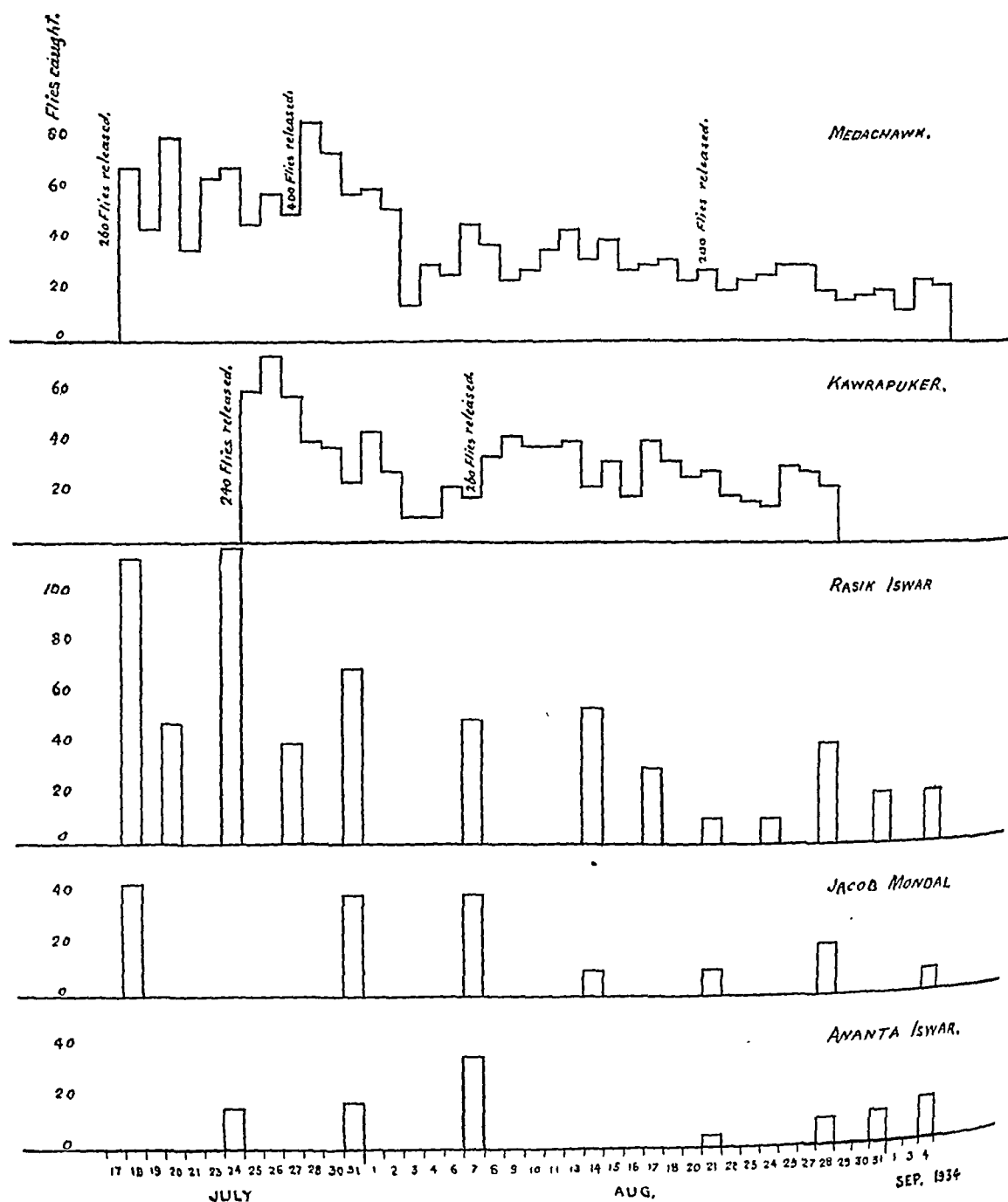
We do not feel justified in drawing any definite conclusions from these observations. They are, nevertheless, very suggestive that a considerable destruction of these midges occurs under natural conditions and among their natural enemies; spiders, of two predaceous species, are responsible to a great extent for the diminution in their numbers.

As these experiments were done in an inter-epidemic period, and only three flies were recovered on the sixth day, the findings are not inconsistent with the incidence of kala-azar at the present time in this area, assuming *P. argentipes* to be the vector. If these findings could be confirmed one reason for the slow spread

of the disease could be explained ; it would also indicate that under natural conditions the inoculation of flagellates into a victim by a large number of infected sand-flies is improbable.

GRAPH.

Showing number of *P. argentipes* collected daily and periodically in five cattle sheds.



The question naturally arises whether one of the factors responsible for an epidemic of kala-azar is an alteration of balance between the insect vectors and their natural enemies. In the laboratory a single spider of the *Attilid* family has been known to consume as many as 30 sandflies in a day. Spiders are not as prolific breeders as many species of insects. It is only after a series of moults, usually about nine in number, that they attain full maturity. Any adverse factor, such as a drought, about the time of their breeding season, which as far as we have been able to determine occurs about twice a year, may result in a considerable diminution of the young members by depriving them of small insects on which they are solely dependent for food. Their cannibalistic tendency also will be more in operation during such a period and cause a still further decrease in their numbers. As many of these species are purely domestic, their value in reducing the numbers of blood-sucking insects which enter dwelling houses is deserving of further investigation.

Our thanks are due to Dr. K. C. K. E. Raja for his help with the figures, to Dr. F. H. Graveley for very kindly identifying the spiders, and to the insect collectors for their co-operation in this work.

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Situation.	Date.	Method of marking.	Number of flies released.	Number of flies captured and marked.	Day of									
					1	2	3	4	5	6	7	8	9	10
1 Medachawk cattle shed.	17-7-34	Fluorescein	260	{ Captured .. Marked ..	68 4	41 0	80 1	36 0		64 1	60 0	48 0	52 0	12 0
2 Kawrapuker cattle shed.	24-7-34	"	240	{ Captured .. Marked ..	68 5	79 1	67 0	40 0		36 0	24 0	44 0	5 0	5 0
3 Medachawk cattle shed.	27-7-34	"	400	{ Captured .. Marked ..	87 5		74 0	58 1	61 1	53 0	15 0	31 0	11 0	11 0
4 Kawrapuker cattle shed.	7-8-34	"	260	{ Captured .. Marked ..	39 10	42 2	38 3	39 0		41 1	23 0	33 0	11 0	11 0
5 Medachawk cattle shed.	21-8-34	"	200	{ Captured .. Marked ..	21 2	24 0	26 0	30 0		30 0	19 0	17 0	11 0	11 0
6 Trivango Mondol house.	29-8-34	"	30	{ Captured .. Marked ..	20 3	13 1	15 0	13 0		13 0	13 0			
7 Trivango Mondol house.	8-9-34	"	75	{ Captured .. Marked ..	17 3		14 1	14 0	13 0	11 0	14 0	6 0	6 0	6 0
8 Kawrapuker cattle shed.	4-9-34	"	150	{ Captured .. Marked ..	37 2	22 0	23 1	13 2		12 0	17 0			
9 Kawrapuker cattle shed.	11-9-34	"	80	{ Captured .. Marked ..	21 3	16 4	13 0	16 0			10 0			
10 Phiren Patro house.	7-9-34	"	75	{ Captured .. Marked ..	1 1		0 ..	2 0	0 ..		0 ..			
11 Medachawk cattle shed.	14-9-34	"	180	{ Captured .. Marked ..	27 8			27 2		17 1	10 0	15 0	11 0	11 0
Carried over ..			1,950	Recovered ..	46	8	6	5	1	3	0	0	0	0

COLLECTION.																Total caught.	Total of marked flies.	Percentage re-covered.	REMARKS.	
10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25					
53	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	519	6	2.3	..
0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
10	10	..	22	18	..	..	..	..	..	..	..	..	..	..	..	}	424	6	2.5	..
0	0	..	0	0	..	..	..	..	..	..	..	..	..	..	..					
6	47	38	23	28	36	..	45	33	41	28	29	33	..	25	27	}	838	7	1.7	..
0	0	0	0	0	0	..	0	0	0	0	0	0	..	0	0					
1	33	..	26	27	18	17	14	30	..	28	23	..	..	..	..	}	531	16	6.1	..
0	0	..	0	0	0	0	0	0	..	0	0	..	..	..	..					
10	12	..	24	22	..	..	..	..	..	..	..	..	..	..	..	}	264	2	1.0	..
0	0	..	0	0	..	..	..	..	..	..	..	..	..	..	..					
8	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	102	4	13.3	..
0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
..	19	..	..	8	..	..	..	..	..	..	..	..	..	..	..	}	116	4	5.3	..
..	0	..	..	0	..	..	..	..	..	..	..	..	..	..	..					
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	124	5	3.3	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	76	7	8.7	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	}	5	1	1.3	..
..	0	..	..	0	..	..	..	..	..	..	..	..	..	..	..					
..	27	30	12	14	19	..	..	..	..	..	..	..	..	..	..	}	181	11	6.1	..
..	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..					
0	0	0	0	0	0	0	0	0	0	0	0	0	0	..	0	3,180	69			

Situation.	Date.	Method of marking.	Number of flies released.	Number of flies captured and marked.	DATE							
					1	2	3	4	5	6	7	8
Brought forward ..			1,950		46	8	6	5	1	3	0	0
12	Phiren Patro house.	25-9-34	Fluorescein	100	{ Captured ..	7	1	0	0	0	0	0
					{ Marked ..	2	0	..	..	..	..	..
13	Trivango Mondol house.	25-9-34	"	100	{ Captured ..	11	9	5	9	..	3	3
					{ Marked ..	5	0	0	0	..	0	0
14	Bonhugli cattle shed.	11-8-34	"	120	{ Captured ..	..	41	..	37	..	..	..
					{ Marked ..	..	0	..	0	..	..	..
15	Bonhugli cattle shed.	29-9-34	Wing puncture.	95	{ Captured ..	25	13	25	28	..	..	28
					{ Marked ..	0	0	0	0	..	..	0
16	Bonhugli cattle shed.	10-9-34	{ Wing puncture 140 Fluorescein 150	{ 290	{ Captured ..	26	48	35	23	33	..	21
					{ Marked {	*5	*2	..	*1	..	..	..
						†1	†3	†1	..	0	..	0
17	Bonhugli cattle shed.	23-9-34	{ Wing puncture 213 Fluorescein 100	{ 313	{ Captured ..	56	47	18	28	21	..	14
					{ Marked {	*7	*5	*2	0	0	..	0
						†2	..	..	..	..	..	..
18	Bonhugli cattle shed.	4-10-34	{ Wing puncture 165 Fluorescein 138	{ 303	{ Captured ..	52	..	28	30	18	27	..
					{ Marked {	*5	..	0	*1	*1	0	..
						†6	..	..	..	..	..	..
TOTAL RELEASED ..			3,271	Recovered	79	18	9	7	2	3	..	..

—concl'd.

LECTION.															Total caught.	Total of marked flies.	Per- centage re- covered.	REMARKS.	
11	12	13	14	15	16	17	18	19	20	21	22	23	24	25					
0	0	0	0	0	0	0	0	0	0	0	0	..	0	0	3,180	69			
0	0	0	..	..	..	..	..	..	..	..	..	..	..	..	}	9	2	2.0	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
2	..	.	..	..	..	..	..	..	..	..	..	..	..	..	}	57	5	5.0	..
0	..	..	..	..	..	..	..	..	..	..	..	..	.	.					
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	78	0	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
22	35	..	27	..	..	..	..	..	..	..	..	..	..	..	}	295	0	..	..
0	0	..	0	..	..	..	..	..	..	..	..	..	..	..					
10	16	..	..	..	..	..	..	..	..	..	..	..	..	..	}	285	13	4.5	Wing punctu 8 = 5.7 per ce Fluorescein 5 = per cent.
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
0	0	..	..	..	..	..	..	..	..	..	..	..	..	..	}	250	16	5.1	Wing punctu 14 = 6.5 per ce Fluorescein 2 = per cent.
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	}	155	13	4.2	Wing punctu 7 = 4.2 per ce Fluorescein 6 = per cent.
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..					
0	0	0	0	0	0	0	0	0	0	0	0	0	..	0	4,309	118	3.6	..	

uncture.
in.

Situation.	Date.	Method of marking.	Number of flies released.	Number of flies captured and marked.							
					1	2	3	4	5	6	7
1 Medachawk cattle shed.	29-9-34	Wing puncture 228 Fluorescein 200	428	Captured .. Marked ..	..	..	..	..	..	..	..
2 New cattle shed	9-11-34	Fluorescein	<i>P. argentipes</i> 100 <i>P. papatasi</i> ? 10	Captured .. Marked ..	30	..	43	45	31	33	14
3 Paran Bagh ..	17-9-35	Fluorescein	<i>P. argentipes</i> 21 <i>P. papatasi</i> 63	Captured .. Marked {	37	30	23	..	..	34	..
4 Other houses close to Paran Bagh.	17-9-35		..	Captured .. Marked ..	47	29	21	..	..	24	0

[illegible]

TABLE II.

Situation.		Full blood meal.	Half or more digested.	Gravid.	Total flies.	Full blood meal.	Half or more digested.	Gravid.	Unfed females.	Males.
Medachawk	..	298	41	93	519	298	41	93	7	80
Kawrapuker	..	57	23	57	446	57	23	57	95	214
Medachawk	..	353	84	203	805	353	84	203	19	146
Kawrapuker	..	166	37	113	508	166	37	113	37	155
Medachawk	..	128	37	67	264	128	37	67	3	29
Trivango ..	..	41	26	18	102	41	26	18	1	16
Trivango ..	..	44	17	18	116	44	17	18	1	36
Kawrapuker	..	32	18	10	124	32	18	10	12	52
Kawrapuker	..	23	3	8	76	23	3	8	4	38
Medachawk	..	71	26	38	181	71	26	38	4	42
Trivango ..	..	24	7	7	57	24	7	7	1	18
TOTALS ..		1,237	319	632	3,198	1,237	319	632	184	826
Percentages ..		56.5	14.5	28.8	..	38.6	10.0	19.7	5.7	25.8

APPENDIX.

Duration of life of the sandfly P. argentipes under laboratory conditions and other statistical data.

BY

K. C. K. E. RAJA.

IN order to form an idea of the longevity of sandflies under laboratory conditions, the following data are available. During the period May 1933 to September 1934, 12,998 laboratory-bred flies took a first feed, and of them 3,337 survived till the third feed. Of these 3,337 flies, 236 were known positives, and records were kept of their order of dying out. Dr. Smith considers that the presence of flagellates in the fly does not in any way shorten its life-history. If the rates of mortality of this small sample of 236 flies is applied to the larger group of 3,337 flies, and if in view of the smallness of the number, the rates are graduated by a third order parabola, before being applied to the larger population (3,337) we obtain the figures shown in the following Table as representing the population of flies at the beginning of each age period given (see also Graph) :—

TABLE.

The ages shown are based on average experience of the numbers of days corresponding to the respective feeds (as given to me by Dr. Smith), in relation to which the enumerations of flies took place.

Age in days.	Population of flies.	Rate of mortality.	Number of deaths.
6.5 to 9.0 ..	3,337	0.41441	1,383
9.0 to 11.5 ..	1,954	0.55100	1,077
11.5 to 14.0 ..	877	0.55193	484
14.0 to 16.5 ..	393	0.50348	198
16.5 to 19.0 ..	195	0.49193	96
19.0 to 21.5 ..	99	0.60356	60
21.5 to 24.0 ..	39	0.92465	36
24.0 to 26.5 ..	3	1.54148	3

We started with 12,998 first fed flies. The first feed usually takes place within 24 hours after emergence from the pupal case. No record was kept of the number of flies which survived for the second feed between the 3rd and 4th day after emergence. The third feed corresponds roughly to 6·5 days and subsequent feeds take place, Dr. Smith considers, approximately at intervals of two days and a half. The following Table gives the percentage of flies that died out within the respective periods indicated, when we started with a population of 12,998 flies :—

Within the first		Percentage that died.
6·5 days	..	74·33
9·0	..	84·97
11·5	..	93·25
14·0	..	96·98
16·5	..	98·50
19·0	..	99·24
21·5	..	99·70
24·0	..	99·98

A fly becomes infective only about the time of the third feed and it may be seen that by that time (6·5 days) only a little over 25 per cent survive. The bearing that this fact may have on the transmission of kala-azar is dealt with elsewhere by Drs. Smith and Mukerjee.

Capture of marked flies.

An examination of the figures relating to recovery of marked flies in Table I reveals the fact that the proportions recovered show a significant difference in the case of some experiments when compared in pairs and that significance emerges when the series of 20 experiments is considered as a whole. But the numbers recovered were always small except in one instance. In all the experiments together 3,271 flies were released and 118 recovered. It has been stated by Drs. Smith and Mukerjee that two species of predaceous spiders, natural enemies of the sandfly, were responsible for producing a certain amount of depopulation among them.

These experiments relate to *P. argentipes* which is known to be less active in its habits than *P. papatasii*. It is possible that the sluggish habits of the former render it more easily a prey to its natural enemies than the latter. An experiment on exactly the same lines carried out with *P. papatasii* gave 15 recoveries when 63 flies were released. Unfortunately, as the Inquiry was closing, further experiments with this species could not be continued. However, a comparison of the rates of recovery in the two cases based on the available data shows that the difference between the rates is significant.

Proportion caught among	<i>P. argentipes</i>	$\frac{118}{3,271}$	= 0.0361.
„ „ „	<i>P. papatasii</i>	$\frac{15}{63}$	= 0.2381.
Difference		..	0.2020.

If we start with the assumption that both (3,271 and 63) are random samples from the same population and that the difference between the two rates may be due solely to chance, our best estimate of probability of recovery is

$$p = \frac{118 + 15}{3,271 + 63} = 0.0399 \quad q = 0.9601.$$

The standard error of difference = 0.0249.

$$\frac{\text{difference}}{\text{st. error}} \quad .. \quad 8.11.$$

Hence it is quite significant.

It may be that *P. argentipes* being less active than *P. papatasii*, the natural enemies were able to destroy the former more easily than the latter. If active habits of the fly interfered with the process of catching, the ratio caught in the case of *P. papatasii* should have been significantly less than in the case of *P. argentipes*. This is not so.

THE TRANSMISSION OF *L. DONOVANI* BY THE BITE OF THE SANDFLY *P. ARGENTIPES*.

BY

R. O. A. SMITH, D.T.M., I.M.D.,
JEMADAR CHIRANJI LAL, I.M.D.,
S. MUKERJEE, D.SC.,

AND

K. C. HALDER, L.M.P.

(From the School of Tropical Medicine, Calcutta.,

[Received for publication, 16th March, 1936.]

THE object of this note is to place on record one more successful transmission of *L. donovani* to a hamster (*Cricetulus griseus*) by the bite of the sandfly *P. argentipes*. The hamster which showed the infection was one of 16 which were given an average of ten known positive feeds each, before they were separated off to complete the incubation period of approximately 16 months.

In another series of 20 hamsters, each hamster was given one known positive feed at the 5th, 6th, 7th and 8th feed of an infected fly. No positive results were obtained in this series.

The multiplication and disposition of the flagellates of *L. donovani* in the sandfly *P. argentipes* being so suggestive of transmission by the 'bite' of the fly, no factor other than host-resistance was cited as the cause of failure in the earlier transmission experiments. The fact that three successful transmissions to hamsters had already been obtained (Shortt, Smith, Swaminath and Krishnan, 1931; Napier, Smith and Krishnan, 1933) was evidence that flagellates capable of causing an infection were inoculated at the bite of an infected sandfly, if not always, then on certain occasions. That the incubation period for the development of the infection was so prolonged—16 months in the hamster after being 'bitten' by infected sandflies—might have been due as well to the smallness of the dose as to a resistance in the victim.

In the search for some factor in the hamster which might have raised its resistance to a small dose of flagellates, it was found that hamsters kept in the laboratory for long periods on a diet of soaked gram and vegetables—either lettuce

TABLE.
Showing transmission experiments in hamsters.

HAMSTERS WHICH HAD EIGHT OR MORE KNOWN POSITIVE FEEDS.				HAMSTERS WHICH HAD 4 TO 7 KNOWN POSITIVE FEEDS.				HAMSTERS WHICH HAD UNDER FOUR KNOWN POSITIVE FEEDS.			
DIED.		SACRIFICED.		DIED.		SACRIFICED.		DIED.		SACRIFICED.	
Serial number.	Incubation period in months.	Result.	Incubation period in months.	Result.	Serial number.	Incubation period in months.	Result.	Serial number.	Incubation period in months.	Result.	Incubation period in months.
2	..	..	17	Positive	1	9	Negative	..	4	..	16
6	..	..	16	Negative	5	..	..	Negative	19	13	..
7	17	Negative	..	..	17	..	..	"	25	..	11
8	..	..	15	Negative	23	12	Negative	..	..	..	..
9	..	..	21	..	24	7	..	..	..	..	..

10	..	20	"	..	..
11	..	20	"	..	..
12	..	19	"	..	..
13	14 Negative	..	..	..	..
14	8 "	..	..	..	..
15	4 "	..	..	..	..
16	3 "	..	..	..	..
18	..	16 Negative	..	..	..
20	..	14	"	..	..
21	..	13	"	..	..
22	..	13	"	..	..

or cabbage—had, when compared with hamsters recently imported from China, a higher adrenal content, when assayed biologically. It was the opinion of the Director, the late Colonel Acton, that an infection with *Leishmania* was not compatible with a high adrenal content. In addition to the low blood pressure and the pigmentation of kala-azar patients being ascribed to adrenal deficiency, he had not seen a case of visceral leishmaniasis in a victim either of diabetes or leucoderma, two diseases fairly common in Bengal, where kala-azar is endemic, and which are often associated with hyper-adrenalæmia.

The only variation made in these transmission experiments, therefore, was in the care and feeding of the animals, which were given a liberal mixed diet and plenty of water. That the general health of the hamsters was influenced by this treatment was reflected by the success obtained in breeding these animals for the first time under laboratory conditions. The results obtained in the attempts to transmit the infection to them were, however, disappointing.

The susceptibility of the hamster to *Leishmania* being so great, it is improbable that the host resistance factor was operative in these or on the previous occasions when this animal was used. Unless the rôle of the sandfly in the transmission of kala-azar be excluded—a procedure which is not justified in view of four successful transmissions secured up to date, as well as the close relation between this insect and the disease, both epidemiologically and biologically—the cause of failure to transmit the infection should be investigated in the sandfly, with special reference to the presence and virulence of the flagellates in the buccal cavities of infected flies, on which the success or otherwise of an infection depends. Better results would possibly be obtained if the laboratory technique was modified, so that more natural methods of breeding and feeding *P. argentipes* were practiced.

The results of the transmission experiments are given in the above Table.

Our thanks are due to Colonel R. N. Chopra and to Dr. J. C. Gupta for very kindly estimating the adrenal content of the hamsters, to Insect Collectors Messrs John A. Day and P. Roy for much work in connection with the feeding of the infected flies on the hamsters.

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FURTHER OBSERVATIONS ON INDIAN RELAPSING FEVER.

Part III.

PERSISTENCE OF SPIROCHÆTES IN THE BLOOD AND ORGANS OF INFECTED ANIMALS.

BY

LIEUT.-COLONEL J. CUNNINGHAM, C.I.E., B.A., M.D.,
Medical Superintendent, The Astley Ainslie Institution, Edinburgh,

AND

ASSISTANT SURGEON A. G. L. FRASER, I.M.D.
(An Inquiry under the Indian Research Fund Association.)

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THE persistence of spirochætes in the blood and organs of infected animals at times other than the recognized attacks has attracted considerable attention, because it introduces problems of the greatest interest and importance connected with immunity and the possibility of cure.

The presence of spirochætes in the blood between the attacks of fever has been noted by many authors (Serjant and Foley, 1914 ; Moselli, 1923 ; and other workers). Persistence for varying periods after the final attack has also been shown repeatedly. Weichbrodt (1920), Todd (1920) and Mayer (1922) have proved the blood to be infective at periods of 42, 32 and 22 days after the last attack respectively. More recently special significance has been attached to the persistence of the organisms in various internal organs, but particularly the brain, and a considerable literature has accumulated around this subject.

Such resistance has been much discussed in regard to its relationship to questions of immunity, resistance to re-infection or super-infection, and the possibility of cure with arsenical preparations.

Ehrlich and Hata (1910), in their early experiments with mice, considered that a daily negative examination of the blood for sixty days, followed by successful re-infection, established the proof of cure. Inability to re-infect demonstrated a latent infection (*immunitas non-sterilans*).

Various authors (Brault and Montpellier, 1914; Plaut and Steiner, 1920; Weichbrodt, *loc. cit.*; Werner, 1924) have noted the presence of spirochaetes in the cerebro-spinal fluid of patients suffering from the disease, and the last named found that the organism persisted long after all fever had ceased, and also after repeated doses of arsenicals. The observations of Kudicke, Feldt and Collier (1924) are of special importance in this connection. Strains isolated by them from the cerebro-spinal fluid at different periods differ from each other and always from those found in the blood.

Buschke and Kroo (1923) first claimed that relapsing-fever spirochaetes may invade the brain substance in mice and drew attention to the significance of such infections. Numerous observers have corroborated these findings. *S. recurrentis*, *duttoni* (including its variants), *hispanica*, and a Manchurian strain have all been incriminated. Such infections may last for long periods [as long as 409 days (Beunders and van Thiel, 1932)] after the blood is free of spirochaetes. The frequency with which these different strains produce brain infections differs very considerably, while individual strains may also show considerable variation in this respect at different times. It would appear from the literature, however, that strains transmitted by the tick are more likely to invade the brain than *S. recurrentis*, although this is not without exception, for Coleman (1934) reports the absence of brain infections so far as the Californian strain investigated by him is concerned.

The significance of these residual brain infections has received much attention. Buschke and Kroo (*loc. cit.*) and Kroo (1926) claimed that the spirochaetes in this situation gained special characteristics, i.e., developed a special neurotropism, that immunity to further infection lasted only as long as the organisms remained in the brain and that they persisted in this situation in spite of treatment. According to Rosenholtz, Owsjannikowa and Treflow (1928) the spirochaetes enter the nervous tissue and live in a kind of symbiotic relation with this tissue (neurobiose). Levaditi and Anderson (1928) believe that such organisms persist in an invisible stage, although Beunders and van Thiel (*loc. cit.*) deny the existence of such a phase. More recently, Tokura (1933) denies the invasion of the brain tissue and considers that the organisms persist in the blood islands of the smaller capillaries resulting from thrombus formation. The existence of a special neurotropism is denied by Prigge (1926) and Prigge and Rothermundt (1928*a* and *b*), who hold that residual infection is the result of a low degree of immunity developed in the host against infection associated with an organism of low virulence. Hiroki (1933) and Toda and Hiroki (1934) oppose this view, however, as they have been able to show that the number of residual infections does not depend upon the virulence of the strains used for experiment.

The relation of residual infections to the continued state of immunity is of considerable importance. Buschke and Kroo (*loc. cit.*) and Kroo (*loc. cit.*) supported the view of *immunitas non-sterilans* originally held by Ehrlich and Hata. Heronimus (1928) and Jackimow (1929) held that immunity to re-infection was due in the first place to the persistence of the brain infection assisted to a varying extent by the development of humoral antibodies. Prigge (*loc. cit.*) and Prigge and Rothermundt (*loc. cit.*) gave pride of place to antibody formation and held that after natural recovery with satisfactory production of antibodies, residual brain infections were rare.

Most observers agree that once spirochætes are established in the brain they resist the action of salvarsan or similar drugs to a greater extent than spirochætes in other situations, and that under such conditions the brain is either incapable of sterilization (Schauder, 1928; and other workers) or is the last organ to be cleared before cure (Schureus and Weisbecker, 1926). Opinion is divided, however, on the power of the drug to prevent residual infections in the first place. Those who favour a special neurotropism for these parasites consider that residual infections occur in spite of treatment (Kroo, *loc. cit.*; Lebedjeva and Ssinjuschina, 1927; Schauder, *loc. cit.*). The opposing school (Tomioka, 1924; Reiter, 1925; Prigge, *loc. cit.*; Johannessohn, 1926) holds that early and energetic treatment will completely sterilize the blood and prevent residual infections even though they may occur when treatment has been delayed or when inadequate. It is difficult to compose the views of the numerous observers on this subject, but the variation in the number of brain infections occurring even with the same strain at different times, which has prompted Rothermundt (1932) to question the value of such experiments as a criterion of the efficacy of any therapeutic substance, has undoubtedly much to do with the differences in the views expressed.

Apart from the work of Kudicke, Feldt and Collier (*loc. cit.*), little or no consideration has been given to the capacity of these organisms for rapid serological variation and its possible bearing on the problems under discussion. This omission seems to us to be fundamental and it is with the object of investigating these aspects of the problem that our experiments were undertaken.

Our investigation has followed the lines of those of other observers, in that we have attempted to prove the presence or absence of spirochætes in the tissues at various periods during and after infection by sub-inoculating a series of animals from the organs of the infected animal and noting the presence or absence of infection in the sub-passages. For the purposes of this investigation the sub-inoculations were carried out on different days corresponding to the periods between the first and second attacks, i.e., the '1st interval', and at various times, early and late, after the second attack, i.e., the '2nd interval'. In different cases sub-inoculations were made from one or more of the following tissues: peripheral blood, heart blood, liver, spleen, brain, and bone-marrow. In the case of the organs not less than half of the emulsified organ was distributed between two to four fresh animals. The sub-inoculations were made intraperitoneally and the blood of the sub-passage was examined under the dark-ground illumination for the following fifteen days. Certain of the animals died within this period from the effects of the inoculations, but, as a rule, there were one or more survivors which enabled conclusions to be drawn for each group.

In addition, the types of spirochæte found were identified and the kinds of antibody present in the blood at the various phases of the disease were estimated where necessary both in the parent squirrels and the sub-passages from them.

Our results have been arranged in four tables. Table I shows the results of sub-inoculations carried out at different times during the '1st interval', i.e., within seven days after the end of the 'first attack'. The experiment divides itself into two series; the first consisting of three squirrels, in which the peripheral blood and organs were used for sub-inoculation, the second, a group of seven animals, where the heart blood only was sub-passaged during life.

In the first series the peripheral blood was proved infective in one out of the three cases, while the organs were proved positive in two out of the three squirrels used for the experiment. These findings coincide with those of other observers already referred to. The type of spirochæte found differed in each case from that which was responsible for the primary attack, and was obviously capable of developing in the body as the serological observations showed; antibodies to the infecting type alone had been developed so far.

In the second series the heart blood of five out of the seven animals was found infective, although no evidence of infection of the peripheral blood could be found by the ordinary methods of examination (i.e., dark-ground illumination). These results are interesting in that they bear out the views that we have already expressed that the mechanism of infection consists essentially of a series of waves of infection by different types of spirochæte, some developing into visible attacks, others remaining latent, and each wave being overcome by specific antibody formation until the infection finally dies out. During the intervals between the visible attacks the spirochætes appear to retire into the internal organs, including the interior of the heart, where the process of development takes place until sufficiently numerous to overflow into the peripheral circulation.

Although practically all the different types of spirochæte we had isolated were represented in the initial infections, the types found in the sub-passages were limited, with only two exceptions, to the types 'A' and 'B', corroborating our opinion that there is a definite urge on the part of the subsidiary types to return to the primary types when opportunity offers (Cunningham and Fraser, 1934).

The second table (Table II) shows the results of sub-inoculations carried out from the blood (in one case, the peripheral blood, in the remainder, the heart blood) and organs of a series of six squirrels at periods immediately following the second attack (first relapse).

These results are entirely different from those obtained from sub-passages carried out from animals during the '1st interval' in that no evidence of living spirochætes could be found in either the blood or the tissues of the various organs, including the brain. This is explained to a great extent by the development of antibodies in the blood to not less than three types of spirochæte, which has been sufficient to counteract any further multiplication of the organisms.

Tables III and IV give the results of a similar series of experiments carried out at much later periods.

In Table III the peripheral blood of sixteen squirrels has been sub-passaged at intervals varying between 163 and 59 days after the end of the first attack. In Table IV sub-passages from the blood and organs of nine animals have been made between 130 and 57 days after the primary infection.

In no case in either series did infection result, proving the sterility of the blood, or blood and tissues, at the particular periods the sub-inoculations were carried out. Examination of the serum at the date of sub-passage once more showed the presence of antibodies to one or more types of spirochæte, more commonly the latter; the titres, while generally lower than those recorded at the end of the attack, being still sufficient to prevent any recurrence or continuance of infection with these particular types, thus causing the infection to die out.

TABLE II.

Showing the results of sub-inoculation of the blood and organs of infected squirrels at a period immediately following the first relapse.

Squirrel number.	TYPE OF SPIROCHETE IN		Day of 2nd interval sub-inoculation performed.	TYPE OF SPIROCHETE FOUND BY SUB-INOCULATION FROM						REACTION OF SERUM TO SPIROCHETAL TYPES ON DAY OF SUB-INOCULATION.						
	First attack.	Relapse.		Peripheral blood.	Heart blood.	Liver.	Spleen.	Brain.	Bone-marrow.	A.	B.	C.	D.	E.	G.	I.
10517	D	AB	1	:						10,240	5,120	—	5,120	—	—	:
10661	C	B	1	:						2,560	1,280	5,120	—	—	—	:
10436	B	A	4	:		:				10,240	2,560	—	—	40	—	:
10437	B	A	4	:						20,480	10,240	—	—	40	—	:
10339	B	AE	5	:						10,240	5,120	—	10,240	1,280	10,240	:
988†	A	B	11	—	:					5,120	10,240	1,280	—	—	—	—

TABLE III.

Summarizing the results of sub-inoculation of the peripheral blood of squirrels which have survived an attack of the disease for various periods previous to sub-inoculation.

TABLE III.
Showing the results of sub-inoculation of the peripheral blood of squirrels which have survived an attack of the disease for various periods previous to sub-inoculation.

Squirrel number.	TYPE OF SPIROCHÆTE IN		Number of days since attack.	Sub-inoculation from peripheral blood.	TITRE OF SERUM TO SPIROCHÆTAL TYPES AT TIME OF SUB-INOCULATION.						
	First attack.	Relapse.			A	B	C	D	E	G	I
7779	E	..	163	—	—	—	—	40	40	—	—
8078	B	..	125	—	160	40	320	—	—	—	80
8097	I	..	124	—	—	—	40	—	—	—	40
8338	C	..	103	—	160	40	320	—	—	—	—
8409	E	..	96	—	80	—	—	640	160	—	—
8579	C	..	82	—	—	40	160	—	—	—	—
8574	E	..	82	—	1,280	320	320	—	—	—	20
8588	G	..	81	—	40	10	640	—	—	1,280	—
8590	I	..	81	—	640	320	—	—	—	40	640
8623	C	..	78	—	—	—	1,280	—	—	—	—
9663	G	C	76	—	10	1,280	80	20	—	640	..
8672	C	..	73	—	40	40	1,280	—	—	—	—
9815	E	..	66	—	—	40	160	—	160	—	..
8769	E	..	64	—	640	1,280	40	—	1,280	40	80
8775	I	..	64	—	640	320	80	—	40	—	40
9208	E	..	59	—	—	—	—	—	5,120	—	—

TABLE IV.

Showing the results of sub-inoculation of the organs of squirrels which have survived an attack of the disease for various periods previous to sub-inoculation.

Squirrel number.	TYPE OF SPIROCHÆTE IN		Number of days since last attack.	TYPE OF SPIROCHÆTE FOUND BY SUB-INOCULATION FROM						TITRE OF SERUM TO SPIROCHÆTAL TYPES AT DATE OF SUB-INOCULATION.						
	First attack.	Relapse.		Peripheral blood.	Heart blood.	Liver.	Spleen.	Brain.	Bone-marrow.	A	B	C	D	E	G	I
8441	A	..	130		:					640	—	40	320	—	—	10
9324	G	:	96	:				:	:	—	80	80	20	—	20	:
9103	I	+	95		:					40	40	—	—	—	—	80
9123	E	:	93		:					—	—	—	—	—	—	—
9157	B	:	89		:					—	1,280	—	—	—	—	20
9170	G	:	89		:					—	—	—	—	—	10	—
9177	E	:	88		:					—	—	—	—	80	—	—
9277	A	:	79		:	:				1,280	1,280	320	—	—	—	:
9992	D	..	57	:					:	20	—	—	2,560	—	—	..

SUMMARY AND CONCLUSIONS.

The conclusions to be drawn from these experiments may be summarized as follows:—

1. Animals infected with *Sp. carteri* maintain spirochætes in the blood and internal organs throughout the interval between the first and second attacks (and would undoubtedly do so between the second and third attacks should a third attack occur). Such latent infections can be demonstrated by sub-inoculation of the blood, particularly the heart blood, or organs taken from an infected animal during this period.
2. On the other hand, 'residual infections', as the term is usually understood, and particularly invasion of brain tissue, do not occur with the Indian louse-borne strain of spirochæte.
3. The spirochæte found during the '1st (or 2nd) interval' by such means always differs in type from the spirochæte initiating the primary attack, and will, in all probability, simulate the type present in the relapse, if a relapse should subsequently occur.
4. The serological examination of the blood carried out at various periods during and subsequent to infection bears out the conclusion previously reached by us that the disease essentially consists 'in most cases of a succession of invasions by different types of spirochæte which vary in intensity and duration; the essential difference between cases which exhibit "single" attacks and those showing "relapses" being only one of degree in that the later attacks are "latent" in the one case and demonstrable by blood examination in the other'.

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Note.—References marked * were consulted in the *Tropical Diseases Bulletin* only and not in the original.

FURTHER OBSERVATIONS ON INDIAN RELAPSING FEVER.

Part IV.

RE-INFECTIONS AND SUPER-INFECTIONS.

BY

LIEUT.-COLONEL J. CUNNINGHAM, C.I.E., B.A., M.D.,
Medical Superintendent, The Astley Ainslie Institution, Edinburgh,

AND

ASSISTANT SURGEON A. G. L. FRASER, I.M.D.
(An Inquiry under the Indian Research Fund Association.)

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THE consideration of re-infection and super-infection raises various questions dealing with the immunity acquired by the primary infection. In the first place the degree of protection produced is of primary importance with regard to re-infection and will almost certainly have a definite influence on a super-infection.

A high grade immunity should resist re-infection with the same strain as long as it lasts. A considerable amount of work designed to test the extent of this resistance has been recorded by different observers.

Ross (1911) (Kenya strain) and Nicolle and Blaizot (1913) (Tripolitan strain) showed that monkeys could be re-infected shortly after recovery with a second inoculation of the same strain. Serjent and Foley (1914) showed that spirochætidal substances protected monkeys one and a half months after recovery, but they were absent from the blood in four months. These observers also held that the immunity acquired by an attack in man was not of long duration. Oliver (1924) held the same opinion, as re-infections in certain of his cases occurred six months after recovery. In our earlier work with the Madras strain (Cunningham, 1925)

re-infections were carried out in monkeys at different times after recovery with a varying amount of success.

With regard to super-infection, Kudicke, Feldt and Collier (1924) showed that it was possible to superimpose an infection during the interval notwithstanding the fact that the first infection was still in existence, and Steiner and Steinfeld (1926) studying the effect of repeated inoculations in the same animal showed that with a mild primary infection it is possible to superimpose a more intense infection by re-inoculation.

The conception of an *immunitas non-sterilans* complicates the question still further, for, under these conditions, resistance to further infection is evidence of the continuance of the primary infection not of an acquired immunity. Indeed, Ehrlich and Hata (1910) considered that a successful re-infection after 60 days' negative examination of the blood was a proof of cure.

As already noted, however, the existence of residual brain infections has raised doubts as to the efficacy of this test, and Kitschenski and Brussin (1926) have pointed out that considerable difficulty may be experienced in deciding whether a re-appearance of spirochætes in such circumstances is due to a true re-infection, a super-infection, or a provocation of an already existing infection.

While the specificity of various strains has been fully recognized (Weichbrodt, 1920; Nicolle and Conseil, 1923) we do not think that sufficient weight has been attached to the variations in type which occur within the same strain in the consideration of these problems. We, therefore, believe that the publication of certain experiments which were devised to show the basic importance of these changes in such cases may serve a useful purpose.

I. RE-INFECTIONS.*

We have already published a detailed study of the course of antibody formation (Cunningham and Fraser, 1935) in infected animals and a further consideration of this question need not detain us. What is of more importance for our present purpose is the question of the length of time such antibodies remain in the blood stream after the primary infection.

In the first table (Table I) the agglutinin content of the serum of five monkeys infected with different types of spirochætes has been recorded at 30 days' intervals for periods extending over a year, and, in most cases, until the agglutinins have finally disappeared from the blood. One of the animals (No. 181A) reacted positively to the infecting type for 570 days, while another (No. 188C) only reached 360 days, and two other cases (Nos. 186B and 185E) became negative at intervening periods. The fifth case (No. 187D), however, showed a relatively high titre (1:320) on the 480th day and would undoubtedly have continued to do so for a longer period than did the other cases had the animal survived. Antibody produced in response to a relapse disappears sooner (No. 181), and secondary agglutinins formed in the course of single attacks are also of shorter duration.

* It must be understood that the serological types of spirochæte referred to in these experiments have been obtained from a single strain of *S. carteri* derived originally from a case of relapsing fever in a human being. For further details as to the methods used in isolating these types, the reader is referred to our previous papers, particularly Part I of this series (*Ind. Jour. Med. Res.*, 22, No. 4, April, 1935).

TABLE I.

Showing the agglutinin titre of the serum of monkeys infected with various types derived from the same strain of S. carteri taken at intervals of 30 days.

Monkey number.	Type of spirochaete in		Titre of serum examined at 30 days' intervals.																			
	First attack.	Relapse.																				
			30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	510	540	570	600
181	A	B }	A	5,120	1,280	640	640	320	320	320	160	160	160	160	160	160	640	160	80	20	0	0
	B		10,240	320	320	80	40	20	20	20	20	20	20	20	20	10	0	0	0	0	0	0
186	B	— }	B	2,560	640	160	40	80	80	80	80	80	320	160	40	10	20	20	..	..	..	..
	A		1,280	640	80	40	40	80	40	20	20	80	20	10	0	0	0	0	..	..	..	..
188	C	— }	C	10,240	1,280	320	320	80	80	40	40	160	80	80	0	0	..	..	..	..	..	..
	B		320	20	20	0	0	0	0	0	10	10	10	0	0	..	..	..	..	..	..	..
187	D	—	D	10,240	2,560	640	320	320	320	640	640	320	320	1,280	640	320	320	320	..	..	..	..
	E		2,560	640	640	160	20	20	20	20	20	80	20	0	0	10	0	10	..	..	..	..

Note.—Each sample of serum was tested to types 'A', 'B', 'C', 'D', 'E', 'G', 'H' and 'I'. Only positive reactions have been recorded in each case.

As far as immunity due to antibody in the blood is concerned, therefore, monkeys infected under these conditions would be incapable of resisting re-infection with the same strain about 19 months after the primary infection and in many cases much earlier.

The success of re-infection carried out at times *when antibody is present in the blood* will depend to a great extent on the size of the infecting dose*, the type of spirochæte used for the re-infection, and the amount and types of antibody present.

Table II gives details of an experiment designed to show the influence of these various factors on subsequent re-infection. A series of squirrels was selected which had been infected from 64 to 163 days previously. The antibody content of the blood was estimated to each type of spirochæte and then re-infection was attempted with the type which showed the *highest* concentration of antibody. The type selected by this means was generally, but not universally, the type used in the primary infection (*see* Nos. 8078 and 8590). In only one case out of nine (No. 7779) was the re-infection successful, although all the controls gave a positive result. In No. 7779 the titre of the serum was positive only in a 1 : 40 dilution which was obviously too weak to resist the re-infection.

The same squirrels were now subjected to a second re-infection with another type of spirochæte selected on the principle that the blood contained *no* corresponding antibody. The same infecting dose of spirochætes was employed as formerly. On this occasion infection followed in each case. It is obvious, therefore, that a successful re-infection with the same strain, when antibody is present in the blood, depends upon the type of spirochæte used, and the types and concentration of antibody in the circulation: re-infections with 'like' (homologous) types are negative, provided that the antibody content is sufficient; those with 'unlike' (heterologous) types are positive.

The effect of the re-infection on the antibody content is also of some interest. Column 9 of Table II shows the estimation of the agglutinin titre at varying periods after the first re-infection (Nos. 8769, 8775 and 8590) and after the second re-infection (No. 8078). In the former case a considerable enhancement of the previous titre has occurred, while in the latter, agglutinins for the 'heterologous' spirochæte used for the re-infection have also appeared, showing that the re-infection is a new and separate entity and is thus more in the nature of a super-imposed infection.

II. SUPER-INFECTIONS.

The possibility of super-imposing a fresh infection on one already in existence will, to a great extent, depend upon the same factors. Kitschenski and Brussin (*loc. cit.*) have recorded the difficulty experienced by them in distinguishing between a re-infection, super-infection and the provocation of an existing infection in these cases.

We are of opinion, however, that precise information as to the course of antibody formation and the type of spirochæte used for the various infections will give the clue to a correct interpretation in the majority of cases.

* The same infecting dose was employed throughout these experiments. Control squirrels were also inoculated to prove infectivity on each occasion.

TABLE II.

Showing the results of re-infections carried out with types of spirochetes selected on the basis of the type of antibody present or absent from the blood.

Squirrel number.	Type of spirochete in first attack.	Number of days after attack.	TITRE OF SERUM AT DATE OF FIRST RE-INFECTION.							Type of spirochete used for first re-infection.	Result.	Type of spirochete used for second re-infection.	Result.	TITRE OF SERUM AFTER RE-INFECTION.							Date of test.
			TITRE OF SERUM AT DATE OF FIRST RE-INFECTION.											A	B	C	D	E	G	I	
			A	B	C	D	E	G	I												
8078	B	125	160	40	320	—	—	—	80	C	—	E	+	160	40	20,480	—	10,240	—	—	10 days after second re-infection.
8338	C	103	160	40	320	—	—	—	—	C	—	D	+	..	..	died	..	..	..	..	..
8579	C	82	—	40	160	—	—	—	—	C	—	A	+	..	..	died	..	..	..	..	..
8623	C	78	—	—	1,280	—	—	—	—	C	—	B	+	..	..	died	..	..	..	..	..
8769	E	64	640	1,280	40	—	1,280	40	80	E	—	D	+	40	80	40	—	5,120	—	—	13 days after first re-infection.
8574	E	82	1,280	320	320	—	—	—	20	A	—	died	..	..	..	..	..	..	..	..	..
8775	I	64	640	320	80	—	40	—	40	A	—	G	+	1,280	40	80	—	20	—	—	13 days after first re-infection.
7779	E	163	—	—	—	40	40	—	—	D	+	died	..	..	..	..	..	..	..	..	..
8590	I	81	640	320	—	—	—	40	640	I	—	E	+	160	160	20	—	—	—	5,120	13 days after first re-infection.

Note.—Control squirrels inoculated at the same time as the experimental animals with each type of spirochete used in the experiment were all positive 24 hours after inoculation.

Tables III, IV and V give examples of super-infections carried out at various stages of the disease, namely, during the 'first attack' (Table IV), the '1st interval' (Table III), and the '2nd interval' (Table V).

In each case the result of super-infections with types of spirochæte 'homologous' and 'heterologous' to those responsible for the original infection is given. For the sake of convenience the results obtained with infections super-imposed during the '1st interval' (Table III) are described first.

In each of the four examples given super-infection has been carried out 48 hours after the end of the first attack. In the first two squirrels (Nos. 10186 and 10170) the primary infection was obtained with type 'G', in the third and fourth squirrels (Nos. 10191 and 10184) with type 'E'. In the first and third animals super-infection was attempted with the 'homologous' type, in the second and fourth with a 'heterologous' type ('A' type in No. 10170 and 'B' type in No. 10184). In each case the serum was examined for agglutinins immediately prior to the second inoculation, and also on the day following, and the results, which are also given in the table, show a rising tide of antibody formation to the spirochæte responsible for the primary infection.

The increase in the titre seems to be especially marked after super-infection with the 'homologous' type (squirrels Nos. 10186 and 10191) which seems to have acted as a stimulant in this case.

With regard to the results of the super-infection, the two squirrels inoculated with 'heterologous' types (Nos. 10170 and 10184) showed visible infections on the day following which were proved to be due to the spirochæte responsible for the super-infection. In the case of the other two squirrels, infected with the 'homologous' types, spirochætes appeared in the blood of the first squirrel (No. 10186) on the third day. These were shown to be mixed 'B' and 'C' types. In the other squirrel (No. 10191) spirochætes appeared on the fifth day. These proved to belong mainly to the 'B' type. No infection with the spirochæte responsible for the super-infection occurred in these two cases. The serological tests carried out during the course of the disease thus showed clearly that the second appearance of spirochætes was in the nature of a relapse to the original infection and that the super-infection had failed to take.

Table IV gives an analysis of the effects of super-infection during the 'first attack'. In this series the primary infection was carried out with 'A' type and the super-infection with 'E' type. By this means the course of the two infections could be followed independently of each other.

In the first squirrel (No. 10788) the super-infection was carried out 24 hours after, and in the second squirrel (No. 10776) 48 hours after, the primary infection. In the first case the two infections appeared to run concurrently, the first attack was not prolonged and the titre of the serum to the two organisms on the third day of the first interval was 1/2560 to both types.

A relapse, due to a third type 'D', appeared in due course.

In the second squirrel the general course of the disease was similar to that in the first squirrel. In this case, however, the infection caused by the second inoculation definitely lagged behind the primary attack, spirochætes being present

Giving examples of super-infections carried out during the 1st interval with 'like' (homologous) and 'unlike' (heterologous) types of spirochaetes.

Serial number.	Squirrel number.	Day of disease →	1	2	3	4	5	6	7	8	9	10	15	20	25	30	35	40
1	10186	Inoculations →							G	↓								
			+	+	+	+	+	+	↓	↓	↓	↓	BC	+	+	+	+	d
									1280	5120								
2	10170	Inoculations →							A	↓								
			+	+	+	+	+	+	↓	↓	↓	A d						
									5120	10240								
3	10191	Inoculations →							E	↓								
			+	+	+	+	+	+	↓	↓	↓	↓	BA	+	+	+	+	↓
									2560	20480								Serum A 5120 B 1280 E 2560
4	10184	Inoculations →							B	↓								
			+	E	+	+	+	+	↓	↓	↓	B	+	+	+	+	+	d
									1280	2560								

Note.—The type of spirochaete present in the blood at various stages has been recorded by substituting the letter denoting the type found for the usual plus sign.

Giving examples of super-infections carried out during the first attack with 'unlike' (heterologous) types of spirochaetes.

E --

Note:--The type of attachments present in the blood at various stages has been recorded by substituting the letter denoting the type found for the usual plus sign.

Giving examples of super-infections carried out during the 2nd interval with 'like' (homologous) and 'unlike' (heterologous) spirochætes.

Serial number.	Squirrel number.	Day of disease →	1	2	3	4	5	6	7	8	9	10	15	20	25	30	35	40
1	10697	Inoculations →	E	+	+	+	+	+	+	+	+	+	↓ Serum E 5120 D 1280	↓ B				
2	10634	Inoculations →	+	+	+	+	+	+	+	+	+	+	+	↓ Serum B 1280 G 5120				
3	10516	Inoculations →	+	+	+	+	+	+	+	+	+	+	+	↓ Serum A 1280 C 2560	↓ B			
4	10701	Inoculations →	A	+	+	+	+	+	+	+	+	+	↓ Serum A 20480 B 640	↓ D	↓ Serum A 2560 B 10240 D 5120	↓ I	↓ Serum A 5120 B 10240 D 10240 I 20	

Note.—The type of spirochæte present in the blood at various stages has been recorded by substituting the letter denoting the type found for the usual plus sign.

in the blood for eight days. A reference to the third squirrel (No. 10660), where the super-infection was delayed until the third day of the primary infection, proves this to be the case, and shows very clearly the true sequence of events.

On the third day, before the introduction of the 'E' type infection, the only type of spirochæte present in the blood was type 'A'. Two days later (i.e., the fifth day of the primary infection and the third day of the super-infection) about $\frac{1}{4}$ of the spirochætes reacted with the 'A' type, and $\frac{3}{4}$ with the 'E' type, serum, showing that the primary infection had nearly run its course while the super-infection was still progressing in intensity. The following day only 'E' type spirochætes were found, and, two days later, when the squirrel unfortunately died, serum, taken on the day of death reacted well to 'A' type, but was negative to 'E' type, showing that the latter infection had not progressed sufficiently to produce antibodies, while the primary infection had definitely reached the '1st interval' stage.

In Table V examples of super-infections carried out during the 2nd interval are given. In the first squirrel (No. 10697) the super-infecting type of spirochæte is identical with the type responsible for the primary infection (type 'E'), and, in the second squirrel (No. 10634), with the type found in the relapse (type 'B').

In both cases antibodies to the infecting types were present in the blood to a marked degree, and consequently the super-infection failed to materialize.

The next two squirrels (Nos. 10516 and 10701) give examples of super-infections carried out with types of spirochæte different from those which appeared in the primary infection or its relapse. In each case the super-infection was immediately successful. Indeed, in the fourth case the squirrel went through a completely new infection which included a relapse, the serum taken from the animal immediately before death exhibiting antibodies to all the four types of spirochæte which had appeared in the blood.

It should be noted, however, that the majority of infections carried out during the 2nd interval are in reality 're-infections' rather than 'super-infections' because second relapses in squirrels infected with *S. carteri* are very rare and it has already been shown that the spirochæte does not persist in the blood or organs once the relapse has come to an end.

These tests show that super-infections in this particular form of the disease follow the same rules as re-infections, and, to a great extent, depend for their success or failure upon similar factors. If successful the two infections run independently of each other, the exact form taken depending upon the stage of the disease chosen for the super-infection.

It is perhaps unfortunate that residual infections were not found in the Indian louse-borne form of the disease because we were thus unable to subject cases of this nature to a similar range of tests with the object of comparing the type of spirochæte present in such cases with those responsible for the primary attack, and also of investigating the extent of the *immunitas non-sterilans* claimed under such conditions by the methods illustrated above.

SUMMARY AND CONCLUSIONS.

Experiments have been carried out with a view to investigating the part played by the different serological types derived from the Indian louse-borne strain of spirochæte in producing 're-infections' and 'super-infections'. The results which have been obtained warrant the following conclusions:—

1. Monkeys infected with the Indian louse-borne strain of spirochæte retain antibodies in their blood for varying periods up to 19 months after the primary infection. With the disappearance of antibody re-infection will always be successful and will not differ in any way from a completely new infection.
2. When antibody is present in the blood the success or failure of re-infection depends upon the size of the infecting dose, the type of spirochæte used for the re-infection and the concentration and types of antibody produced by the primary infection.
3. Under ordinary conditions of dosage re-infection will fail when sufficient antibody to the type of spirochæte used for the re-infection is present in the blood. When antibody to the re-infecting type of spirochæte is absent or insufficient re-infection can readily be obtained.
4. Re-infection, whether carried out with 'homologous' or 'heterologous' types of spirochætes, produces a rapid and considerable increase in the corresponding antibody content of the blood.
5. Super-infections depend for their success or failure upon similar factors.
6. When antibodies derived from the primary infection are circulating in the blood (i.e., at periods subsequent to the first attack) super-infections carried out with ordinary infecting doses are only successful with 'heterologous' types of spirochæte. 'Homologous' types of spirochæte fail to infect under such conditions.
7. A successful super-infection runs its course as a separate infection which can be distinguished from the different stages of the primary infection by appropriate serological tests even when the infection has been super-imposed during the 'first attack'.
8. The clinical appearances which result from a super-infection depend upon the particular stage of the disease chosen for the super-infection.

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TWO EASY METHODS OF TRANSPLANTING TUBERCLE BACILLI DIRECTLY FROM SOLID TO LIQUID CULTURE MEDIA.

BY

M. B. SOPARKAR, M.D.

(Inquiry under the Indian Research Fund Association.)

(From the Haffkine Institute, Bombay.)

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In the study of the biology of the tubercle bacillus, it is often necessary to observe the character of growth of different strains of this organism on liquid media.

One of the characteristics of this bacillus is that on liquid media its growth occurs on the surface. It is important, therefore, in sowing broth cultures, that the seed used for inoculating should remain floating, for, should it sink, no satisfactory growth would occur.

Successful surface transplantation can easily be accomplished from one broth culture to another by gently lifting with a platinum loop a piece of the surface film from an old culture and lightly depositing it on the surface of fresh broth; but this is not so easy when a strain is sown for the first time on broth from a solid medium.

Perhaps some success may be attained with human tubercle bacilli which often produce on solid media a dry scaly growth which can be removed and lightly laid on the surface of broth on which it remains floating and continues to grow. But this is not always successful, for sometimes the growth sinks and it is more difficult with moist growth such as that of the bovine tubercle bacillus or the slimy growth of the avian tubercle bacillus. Various methods have been tried to overcome this difficulty with more or less success.

Cubes of solid medium with the thickness corresponding to the depth of the fluid have been placed in the medium and sown with cultures, or pieces of cork have been used to support pieces of agar sown with culture on the broth.

Cobbett (1917) recommends the introduction of a small piece of cotton-wool into the broth flask before sterilizing. The wool swells up and extends to the surface and forms a sufficient support for the bacilli which are planted upon it.

Eastwood and recently Lutz (1925) have worked with a combined solid and liquid medium. In this method agar or serum is solidified in a slope in a conical flask and some broth is afterwards added to the bottom; when the surface of the solid medium is planted, the growth gradually extends and creeps over the surface of the broth from which it can be lifted off for subsequent broth cultures.

The methods devised by the writer which have been found very useful and satisfactory are described below:—

The essence of the first method is to keep the seed culture floating on the surface of the broth by placing it on a strip of filter or other absorbent paper and resting the latter on a piece of vaselined paper or other light material. This keeps floating on the broth and supports the filter-paper with the seed culture and growth is favoured by the diffusion of the medium through the filter-paper, the projecting ends of which remain in contact with the medium.

The details are as follows:—

A piece of ordinary paper about 1/10 mm. in thickness and of convenient size, say, about four inches long and two inches broad, is soaked in hot vaseline at a temperature of about 140°C. for about half a minute and drained and the excess of vaseline is wiped off from the surface.

Small pieces, about three-fifths of an inch square, are cut from this and are distributed, one in each, in test-tubes of about three-fourth inch diameter. The exact size of the pieces is of no great consequence provided they are only slightly smaller than the diameter of the test-tube in which they are kept, so that they are capable of being inserted with ease and at the same time are sufficiently large to leave an adequate space between the under-surface of the paper and the wall of the test-tube for a free play of the platinum loop for the purpose of removing the paper (Plate XXIV, fig. 2).

Into the same test-tubes are further placed pieces of ordinary filter-paper, one in each, cut into narrow strips about 1/6 to 1/5 inch wide and of such length that when laid across the vaseline-paper their ends extend about 1/8 to 1/4 inch beyond either edge of the latter. These tubes are sterilized in the autoclave at 15 lb. pressure for one-half hour.

Several such tubes, each containing a piece of vaseline-paper and a strip of filter-paper, may be prepared, sterilized, and kept ready for use whenever required.

The technique is simple. Firstly, by a little manipulation the filter-paper strip is made to lie lengthwise across the centre of the piece of the vaseline-paper in the test-tube without opening it (Plate XXIV, fig. 1). Secondly, with a 5 mm. platinum loop, a drop of sterile broth or salt solution is placed on the filter-paper which makes it stick to the vaseline-paper on which it is lying.

Thirdly, a loopful of culture to be inoculated is removed from the solid medium and is placed in the centre of the filter-paper. A young actively growing culture is always preferable.

Lastly, the whole assemblage, i.e., the vaseline-paper with the filter-paper and the culture, is carefully lifted and removed from the tube with the aid of the same platinum loop inserted under the vaseline-paper and is laid on the surface of the

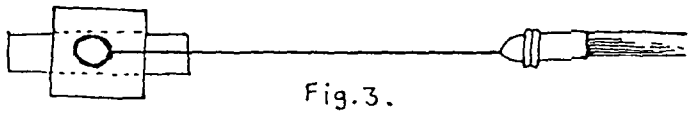


Fig. 3.

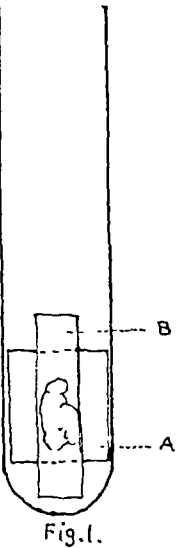


Fig. 1.

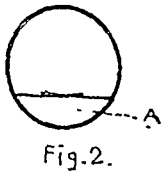


Fig. 2.

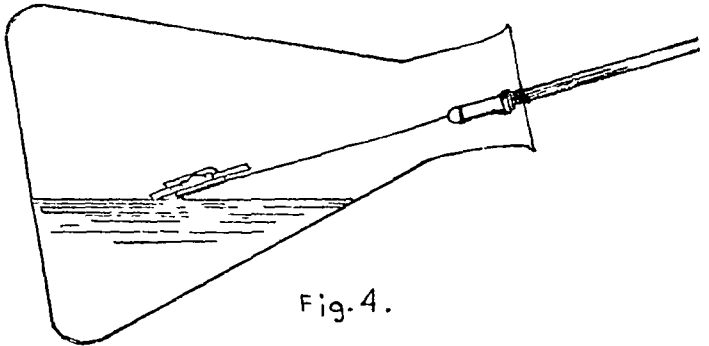


Fig. 4.

- Fig. 1. Tube containing vaseline-paper (A) and a strip of filter-paper (B) lying over it.
 „ 2. Cross-section of a tube with the vaseline paper inside showing space (A) between its under-surface and the wall of the tube.
 „ 3. The vaseline-paper and the filter-paper strip with culture lifted up with the aid of a platinum loop. The capillary attraction of the drop of liquid makes the paper stick to the loop and prevents it dropping off.
 „ 4. The paper with the culture being laid on the surface of the broth.

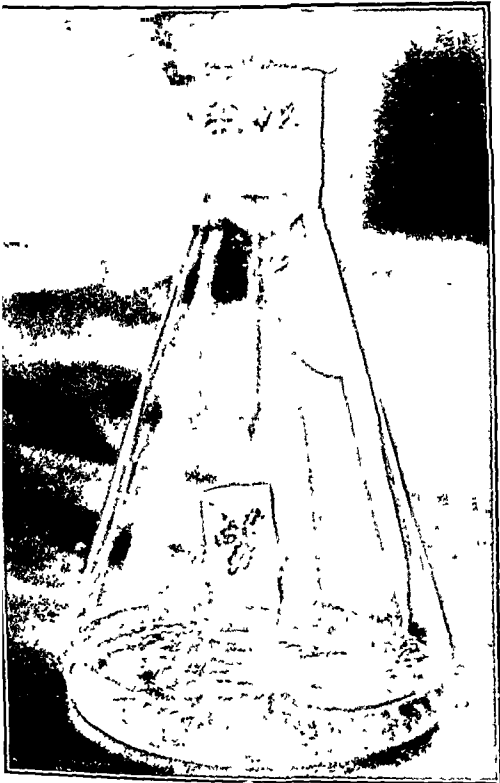


Fig. 5.

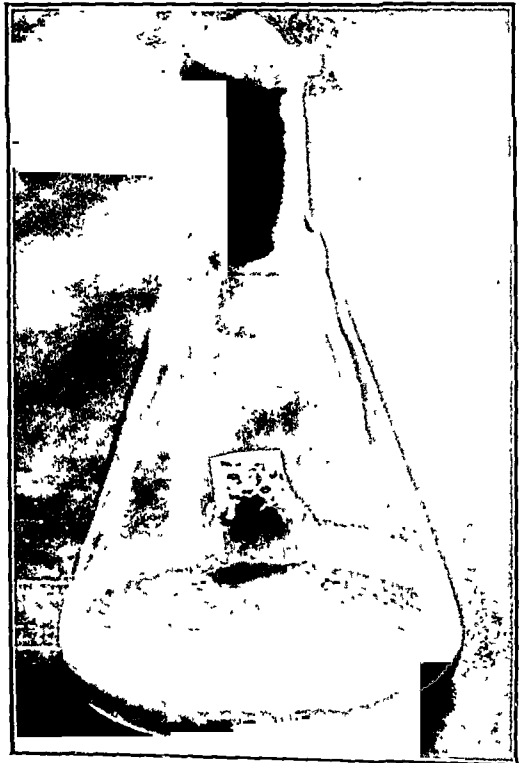


Fig. 6.

- Fig. 5. Conical broth flask showing a piece of filter-paper with the culture spread over it stuck to the inside of the wall of the flask with its lower end below the level of the broth. The growth is seen already commenced in the form of a thin surface film on the broth.
 „ 6. A similar flask after three weeks more of incubation. The whole surface is seen covered with a thick wrinkled growth.



Fig. 7. Rows of flasks inoculated with different strains of tubercle bacilli. The culture on filter paper is seen floating on the broth (before incubation).

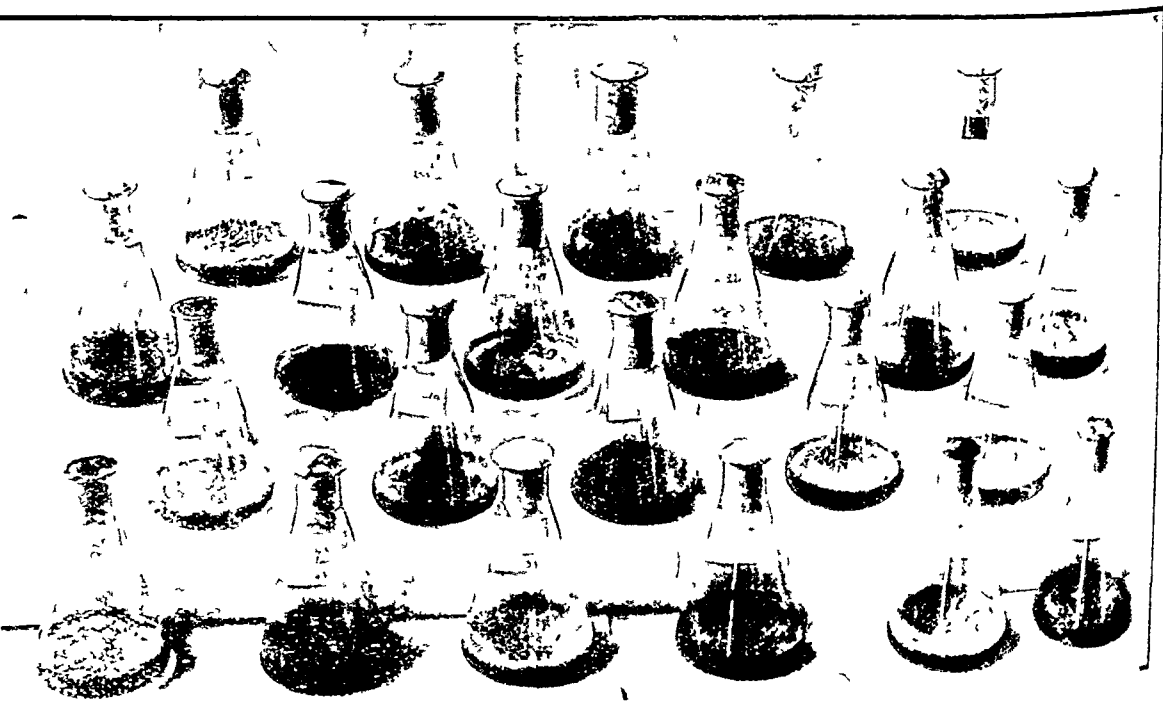


Fig. 8. Rows of flasks after four weeks' incubation, several of which are showing profuse surface growth.

broth by gently touching it with the paper and then lowering the end of the platinum loop beneath the surface of the broth, the flask being kept tilted nearly to a horizontal position for the purpose (Plate XXIV, fig. 4). If the platinum loop which is inserted underneath the vaseline-paper is charged with a drop of broth or salt solution, it will make the paper stick to the loop more firmly than otherwise and the chances of its dropping off the loop in the process of transfer are lessened (Plate XXIV, fig. 3). Several such flasks may thus be inoculated with different cultures and they are then gently removed without shaking and placed in the incubator.

The vaseline-paper continues to float on the surface and supports the filter-paper strip on which is deposited the seed culture and the extended ends of the latter allow, by their contact with the medium, its diffusion over the surface of the paper and promote growth which in course of time extends beyond the paper and ultimately covers the whole surface of broth.

If the filter-paper with the culture after being placed on the surface of the broth should happen to sink through some accident the culture can still be retrieved, provided, of course, it is still sticking to the filter-paper and is not washed off its surface, by pulling up with the aid of a long stout platinum wire loop the sunken filter-paper strip with the culture along the inner side of the flask and keeping it at such a level that the lower end of the strip remains in contact and about $1/8$ to $1/4$ inch below the level of the broth and the culture remains just above it. The result then resembles the second simpler method to be presently described. The other obvious remedy would naturally be to float another filter-paper strip with the culture in the same flask to replace the one that has sunken. More than one such strip may be floated in the same flask to give for each a separate island of growth. These islands will be scattered over the surface and will form several nuclei for fresh growth.

Stableforth (1929), describing the character of cultures of Johne's bacillus on broth, observes 'Surface cultures are difficult to start; usually some agent is necessary to enable the seed material to float and *yet be in contact with the medium*' (*italics* not in the original). This method fulfils these conditions. It has again several other features. It is simple and needs no elaborate preparations and can be carried out even in a small laboratory. The manipulations are easy and sterile conditions can be readily controlled.

Once the papers are kept ready, and they can be sterilized and stocked until needed, any number of cultures of growth of any description and consistency, whether scaly, moist or even slimy, can be readily transplanted directly from the solid to liquid media at any time without much preliminary preparation.

This method has been in use in our laboratory for some years and has proved very satisfactory.

Recently the writer has devised another and a still simpler method:—

In this method, the strip of filter-paper with the seed culture spread over its surface, instead of being floated over the broth, is stuck to the inner side of the conical flask containing the broth at such a level that the lower end of the paper strip remains in continuous contact and below the level of the broth. The wet strip with the culture thus remains stuck to the wall of the flask and the supply of

the medium to the seed through absorption by the filter-paper favours growth which gradually spreads over the surface of the liquid.

The details of the method are as follows :—

Ordinary filter-paper, about 0·15 mm. in thickness (a somewhat thicker paper is preferable), is cut into strips about three-fifth inch broad and about one-and-a-half inches long. The proper width of the strip is of importance for it should be only slightly smaller than the diameter of the tube in which it is kept to allow the platinum loop to be inserted with freedom under the paper for the purpose of its removal. If the strip is too narrow it will stick to the side of the tube when moistened and cause difficulty in its removal. These strips are distributed singly in test-tubes of about three-fourth inch diameter, sterilized in the autoclave and stored for use. When a broth culture is to be made one of these tubes is taken, the filter-paper is shaken and brought to near its mouth and is moistened with a drop of broth or salt solution but not so much as to make it stick to the sides of the tube. The seed culture from the solid medium is now placed in the centre and upper portion of the strip. By means of a thick platinum wire loop charged with a drop of salt solution and inserted under the paper the strip is then lifted up, removed from the tube and inserted in the flask to be inoculated and with a little manipulation made to stick to the inner side of the flask above the level of the fluid. The final position of the paper is then adjusted by means of the same thick platinum loop, so that a short length (about $1/8$ to $1/4$ inch) of its lower end remains in contact and below the level of the fluid ; the object being to maintain a continuous contact with a view to feeding the culture with the medium, the deposited culture being kept just above and touching the fluid. A rigid platinum wire (about 0·7 mm. to 0·8 mm. in thickness) is preferable for these manipulations as the one in common use easily bends. These manipulations are easily carried out in a conical flask but not so in a rounded or bulging flask for which the first method proves more convenient.

This method besides being simpler has a further advantage in that much less care is necessary than in the first method in handling and carrying the flasks to the incubator after inoculation to prevent the culture from sinking, as in this method the culture is no longer floating but is sticking to the side of the flask and if the flask is kept tilted in the opposite direction while being carried, so that the medium gravitates to the opposite side of the paper, the culture remains undisturbed.

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THE NATURE OF IMMUNITY RESULTING FROM T. A. B. INOCULATION.

A STUDY OF 'O' AGGLUTININS IN THE SERA OF INOCULATED HUMAN SUBJECTS AND THEIR IMMUNOLOGICAL SIGNIFICANCE.

BY

CAPTAIN S. S. BHATNAGAR, M.D., PH.D., M.R.C.P., I.M.S.,

ASSISTANT SURGEON J. F. FREEMAN, D.T.M., I.M.D.,

AND

JEMADAR C. S. DHILON, I.M.D.

(From the District Laboratory, Razmak.)

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SINCE the Great War, prophylactic immunization in man against *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B has come to be practised almost universally. This procedure is dictated by the favourable results of statistical evaluation on the protective effects of T. A. B. inoculation as reflected in the rate of incidence and the case mortality rate of infections by these organisms (Leishman, 1921).

Immunological studies on the nature of immunity resulting from T. A. B. inoculation have given contradictory results. Felix (1924), Burnet (1924), and Stuart and Krikorian (1928) contended that the agglutinins produced in the sera of inoculated persons were almost entirely floccular in character—the 'H' agglutinins. These observations would annul the value of T. A. B. inoculation, since it has been demonstrated by immunity reactions, such as active immunization (Arkwright, 1927; Topley, 1929; Schütze, 1930), bactericidal action (Felix and Olitzki, 1926), and phagocytosis (Bhatnagar, 1935), that in the case of flagellated bacilli, as exemplified by the typhoid-paratyphoid group of organisms, the important factors from the point of view of immunity are the 'O' antigens and their corresponding antibodies. Their contentions have, however, been challenged, chiefly as they

relate to the diagnosis of enteric infections by the 'qualitative receptor analysis' method (Felix, *loc. cit.*) by various workers (Gardner, 1929; Whitehead, 1930; Smith, 1932; Mudd, 1932; and other workers). Lately, Giglioli (1933), Dulaney *et al.* (1933), and Dennis and Berberian (1934) have shown that in human subjects both 'O' and 'H' antibodies result from T. A. B. inoculation.

The present investigation was undertaken to study the 'O' agglutinins in the sera of inoculated persons and to assess their immunological significance. The 'H' agglutinins did not form part of the experiments to be described, since it has been shown, in the light of recently acquired knowledge of the antigenic structure of bacteria, that the flagellar 'H' antigens and the antibodies that react with them are of no protective value in active and passive immunization.

The sera were collected from a population which is inoculated every 18 months with two doses (0.5 c.c. and 1.0 c.c. at 10 days' interval) of a T. A. B. vaccine prepared by the Central Research Institute, Kasauli, to the following specifications:—

- (1) The organisms are grown on agar, heat killed (53°C. one hour) and carbonized (finished product adjusted to 0.5 per cent).
- (2) The strains employed are: (a) the classical Rawling's and (b) a recently isolated Indian 'Vi' strain. Both these are added in equal parts. (c) *B. paratyphosus* A (Mears) and (d) *B. paratyphosus* B (Rowlands). All these strains are rendered as 'smooth' as possible before use by broth passage, repeated plating and selection of colonies.
- (3) The vaccine as issued contains per c.c.:—

<i>B. typhosus</i>	1,000 millions.
<i>B. paratyphosus</i> A ..	750 "
<i>B. paratyphosus</i> B ..	750 "

TECHNIQUE.

1. Agglutination tests.

Living bacteria grown 18 to 24 hours on agar slopes, prepared from fresh meat bouillon, and suspended in fresh normal saline, were employed for these tests. One drop of such a suspension, twenty times as thick as that used for this test according to Dreyer's technique, was added to the serum dilutions contained in a total volume of 1 c.c. in tubes with a uniform internal diameter. The tubes were incubated for two hours at 37°C. and left for a further 22 hours at room temperature. The final reading was then taken.

The strains employed for these tests were Ty 901 (O901 and H901) (Weil and Felix, 1920), Watson (Perry, Findlay and Bensted, 1933a), *Paratyphosus* A (OAK) obtained from the Enteric Laboratory, Kasauli, and *B. paratyphosus* B—OB—(Schütze, *loc. cit.*).

All the strains were grown at 37°C., sub-cultured daily and kept under permanent control for smoothness by plating once a week and picking out perfectly smooth colonies for further sub-culturing. In addition to normal saline control

It will be noted that the 'Vi' antibody was detected to a titre of 1 : 50 in the sera of two out of fifty individuals. Both of them gave a history of having suffered from a 'prolonged fever' six months and ten months ago respectively. As Felix, Krikorian and Reitler (1935) have recently drawn attention to the 'possible relationship between 'Vi' agglutinins and the diagnosis of carrier condition, these two individuals were tested carefully for the presence of the enteric group of organisms in their stools and urine. The results were, however, negative.

It was noticed that the figures reported in Table I, what may be termed the normal 'O' agglutinins in a random sample of population, were very much higher than those recorded by observers in other countries and in India (Gardner and Stubington, 1932; Amzel *et al.*, 1934; Lewin, 1934; Bole, 1935; Pasricha *et al.*, 1936; and other workers). An explanation of this discrepancy was, therefore, sought.

Two differences were evident in the technique for agglutination, namely, (1) while live organisms were used by us, killed and preserved emulsions were employed by most other workers, and (2) our tests were set up in tubes of wide internal diameter (1.1 c.c.) with a round bottom. So far as can be judged by the study of published reports, Dreyer's tubes were used by others.

A comparative series of tests were, therefore, carried out on 44 normal human sera to elucidate whether there was any difference due to these two factors.* The results obtained with 10 sera are incorporated in Table II. The other sera gave figures of a similar order. They are not included here on account of economy of space.

TABLE II.

The agglutinability of B. typhosus by normal human sera.

Normal human sera.	AGGLUTINATION OF STRAIN 0901 OF <i>B. typhosus</i> .		
	Live.	KILLED AND PRESERVED	
		in tubes as described in the text.	in Dreyer's tubes.
1	250	150	30
2	200	150	50
3	200	100	25
4	125	75	15
5	100	50	Nil
6	75	45	15
7	75	50	Nil
8	50	30	Nil
9	50	25	Nil
10	25	Nil	Nil

* These tests were carried out by the senior author in collaboration with Lieut.-Colonel R. F. Bridges, R.A.M.C., Officer-in-charge, Enteric Laboratory, Kasauli, by kind permission of the Director of Medical Services in India.

scrupulous cross-examination, any previous history of T. A. B. inoculation was excluded. The results are recorded in Table I:—

TABLE I.

'O' agglutinins for *B. typhosus*, *B. paratyphosus A*
and *B. paratyphosus B* in the sera of
50 uninoculated individuals.

Type of antibody.	Titre of antibody.	Number of individuals.
T. O. {	25	5
	50	12
	100	32
	200	1
A. O. {	25	37
	50	12
	100	1
	200	..
B. O. {	25	12
	50	36
	100	2
	200	..
'Vi' {	10	..
	25	..
	50	2
	100	..

Note.—The figures represent the denominators of titres, i.e., highest dilution of serum in which a partial (+) agglutination was observed by the naked eye according to the following scheme:—

- +++ Plentiful sediment, supernatant fluid clear.
- ++ Plentiful sediment, supernatant fluid turbid.
- + Small sediment, flakes in supernatant fluid.

Observed with 10 × lens { $\pm$ Very small flakes in supernatant fluid.
 $\pm$ Trace.
 $\pm$ Faint trace.

It is seen that all the sera examined possessed the 'O' antibody, for the three types of organisms under consideration, to a titre varying from 1:25 to 1:200. The agglutinins for *B. typhosus* and *B. paratyphosus B* were found to be present in a much higher proportion than those for *B. paratyphosus A*. The variation in the sensitivity of the organisms employed for agglutination may account for these differences to a certain extent. The comparative low agglutinogenic capacity of *B. paratyphosus A*, demonstrated in another communication (Bhatnagar, Freeman and Gera, 1937), and its bearing on the presence of normal agglutinins for this organism will be discussed later.

TABLE III—concl'd.

Type of anti-body.	Titre of anti-body.	INTERVAL AFTER T. A. B. INOCULATION.			
		3 months.	6 months.	12 months.	18 months.
A. O.	25	—	1	4	5
	50	—	1	—	11
	100	3	9	17	8
	200	9	7	4	1
	300	10	7	—	—
	500	3	—	—	—
	750	—	—	—	—
B. O.	25	1	1	6	7
	50	—	1	2	10
	100	2	5	7	7
	200	4	4	7	1
	300	5	6	3	—
	500	9	7	—	—
	750	—	1	—	—
'Vi'	1,000	4	—	—	—
	10	—	Total number of sera examined for 'Vi' antibody = 100.		
	25	4			
	50	1			
	100	—			

It will be seen that 'O' agglutinins for all the three members of the typhoid-paratyphoid group of organisms were found to be present in the sera of all the individuals tested, the titre varying from 1 : 25 to 1 : 1,500. As noted already amongst the uninoculated, so also here in the case of inoculated persons, *B. typhosus* and *B. paratyphosus* B registered higher content of 'O' agglutinins as compared with *B. paratyphosus* A.

A definite time relationship between the titres of all the three types of agglutinins and the date of inoculation are evident from a perusal of Table III. The titres fall as more and more time elapses after inoculation, so that at the end of 12 months they, more or less, approximate to those which have been found to exist amongst the uninoculated individuals (*vide* Table I).

Out of 100 sera examined in this series, the 'Vi' antibody was found to be present in 5 cases to a titre of 1 : 25 (4 cases) and 1 : 50 (one case). On inquiry, all these individuals gave a history of having suffered from a 'prolonged fever' during the last two years.

THE DEVELOPMENT OF 'O' AGGLUTININS IN RESPONSE TO T. A. B. INOCULATION.

The sera of 50 persons, who were due for T. A. B. inoculation, i.e., 18 months after the last course, were collected just before the first dose of the new course and

It will be seen that the live organism proved to be more sensitive than its killed and preserved emulsion. The difference, however, was not such as to justify the high readings reproduced in Table I.

On the other hand, when 'O' agglutination was carried out in Dreyer's tubes and those used by us, the difference between the two readings was very significant. In the latter type of tube, the unagglutinated organisms deposit as a dense circular mass in the centre of the round bottom, while the agglutinated bacteria come down as small granular particles which settle all round the lowest part of the tube. It is thus easy to differentiate between the agglutinated and the non-agglutinated sediment either by the naked eye or with the help of a magnifying lens (10 ×) depending upon the amount of agglutination. Confirmation of this fact is obtained by shaking the tube when the unagglutinated organisms disperse as a homogeneous opacity while the agglutinated granules float in the opaque suspension as such.

Compared with this method, agglutination carried out in Dreyer's tubes suffers from the defect in that it is impossible to say how much of the sediment consists of agglutinated organisms and how much is a pure deposit of non-agglutinated bacteria.

Differences of a similar nature were noticed when normal human sera were titrated for their 'O' agglutinin content against *B. paratyphosus* A and *B. paratyphosus* B. That agglutination in these tubes was not of a non-specific nature was eliminated by absorption tests. It must, however, be stated that when high titre immunized rabbit sera were tested side by side in the two types of tubes, the difference in the titres obtained was much less.

THE PRESENCE OF 'O' AGGLUTININS IN THE SERA OF INOCULATED HUMAN SUBJECTS.

Twenty-five sera of each were collected at random from persons who had been inoculated 3 months, 6 months, 12 months, and 18 months previously and titrated for the presence of 'O' agglutinins. The results are reproduced in Table III:—

TABLE III.

'O' agglutinins at different intervals after T. A. B. inoculation.

Type of anti-body.	Titre of anti-body.	INTERVAL AFTER T. A. B. INOCULATION.			
		3 months.	6 months.	12 months.	18 months.
T. O.	25	—	—	—	—
	50	—	—	1	4
	100	1	—	5	10
	200	1	4	11	9
	300	—	—	8	2
	500	10	18	—	—
	750	8	2	—	—
	1,000	4	1	—	—
	1,500	1	—	—	—

TABLE IV—*concl'd.*

Number.	TITRE OF ANTIBODIES BEFORE T. A. B. INOCULATION.				TITRE OF ANTIBODIES AFTER T. A. B. INOCULATION (25 DAYS AFTER SECOND DOSE).			
	T. O.	A. O.	B. O.	'Vi'	T. O.	A. O.	B. O.	'Vi'
25	200	100	100	0	750	200	1,000	0
26	100	50	50	0	500	300	500	0
27	200	100	100	0	200	200	200	0
28	100	50	100	0	500	500	1,000	0
29	300	100	50	0	1,500	300	50	0
30	100	50	100	0	1,000	200	1,000	0
31	100	25	50	0	200	100	200	0
32	200	200	100	0	200	200	100	0
33	50	100	100	0	1,000	200	500	0
34	50	50	100	0	500	300	1,000	0
35	100	100	50	0	500	200	500	0
36	200	25	50	0	1,000	100	500	0
37	100	100	50	0	500	300	1,000	0
38	100	25	50	0	1,000	200	750	0
39	50	50	100	0	500	300	500	0
40	200	50	50	0	1,500	200	500	0
41	200	25	50	0	500	200	500	0
42	200	100	100	0	750	300	750	0
43	300	25	100	0	500	300	1,000	0
44	100	50	50	0	1,000	100	300	0
45	50	50	50	0	500	200	500	0
46	100	100	100	0	1,000	300	500	0
47	100	200	100	0	500	200	1,000	0
48	300	50	50	0	1,000	200	500	0
49	200	100	100	0	750	300	1,000	0
50	100	100	50	..	1,500	200	500	0

Note.—Titre '0' equals a negative result in dilution 1 : 10.

It was considered that the development of 'O' agglutinins will be demonstrated better if the titres of this antibody, before and after T. A. B. inoculation, were shown, side by side, in all the sera examined. This procedure, however, resulted in a lengthy table. An analysis of the figures shown therein was, therefore, made and is reproduced in Table V :—

TABLE V.

'O' and 'Vi' agglutinins before and after T. A. B. inoculation.

Type of antibody.	BEFORE T. A. B. INOCULATION.		AFTER T. A. B. INOCULATION.	
	Titre.	Number of individuals showing the particular titre. (Total 50).	Titre.	Number of individuals showing the particular titre. (Total 50).
T. O.	50	10	200	5
	100	19	500	20
	200	16	750	7
			1,000	12
	300	5	1,500	6
A. O.	25	8	100	7
	50	17	200	25
	100	19	300	14
	200	6	500	4
B. O.	25	2	100	2
	50	21	200	7
	100	22	300	4
	200	5	500	23
	..	..	750	2
	..	..	1,000	12
'Vi'	10	—	10	—
	25	—	25	—
	50	—	50	—
	100	—	100	—

It was next decided to test the production of 'O' antibody in response to primary T. A. B. inoculation. Twenty-five sera were examined in this connection. The results are included in Table VI. It will be seen that while a considerable amount of 'O' antibody is elaborated for all the three types of organisms, the height of the titres attained, however, falls significantly short of that obtained when the same procedure is adopted in the case of those who have been inoculated before (*cf.* Tables V and VI).

To test that these differences were not due to the particular type of vaccine employed, 10 sera were collected from individuals who had been inoculated six

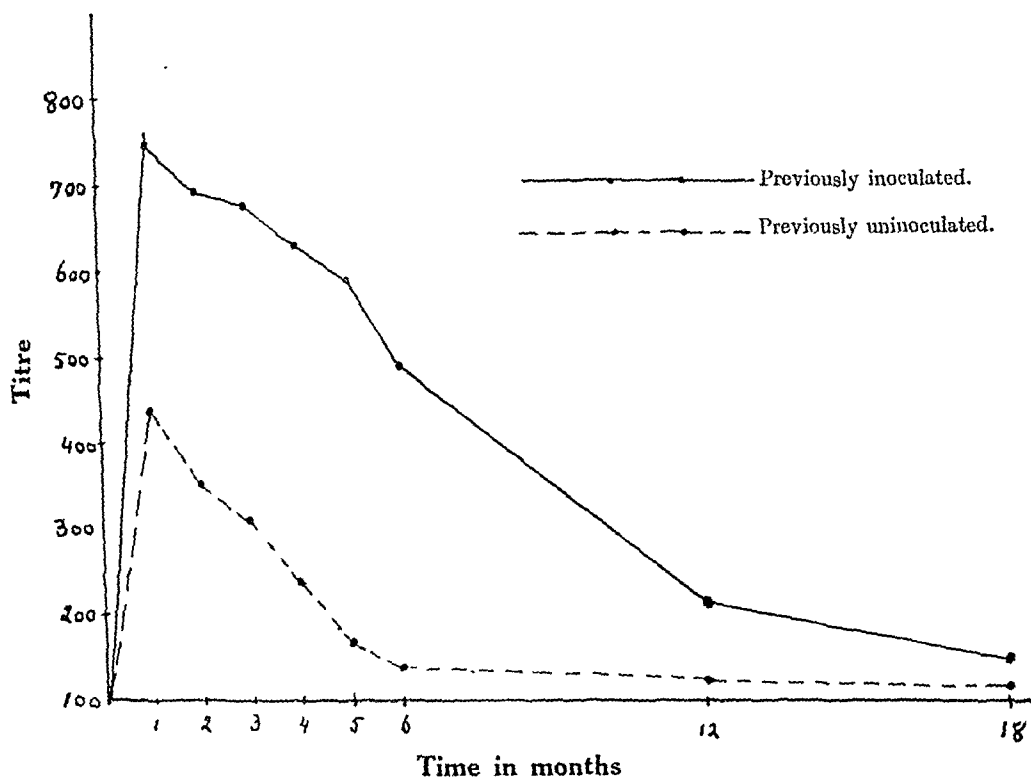


Fig. 1. The production of 'O' agglutinins in human subjects: showing difference in response to primary and subsequent T. A. B. inoculation.

months previously in England with a T. A. B. vaccine prepared by the Royal Army Medical College, Millbank, London, and referred to in various communications by Perry, Findlay and Bensted (1933*a*, *b*; 1934*a*, *b*). Identical results were obtained, the agglutinins being as low six months after primary inoculation with Millbank vaccine as with Kasauli vaccine.

The fall of T. O. agglutinins was next studied in the two types of individuals (primary inoculation versus subsequent inoculation) by bleeding them every month up to six months. The results are charted in Fig. 1 in terms of average titres

TABLE VI.

'O' agglutinins after primary T. A. B. inoculation. Inoculated 6 months previously, 20. Inoculated 12 months previously, 15. Inoculated 18 months previously, 15. Total number examined = 50.

Type of antibody.	Titre of antibody.	INTERVAL AFTER PRIMARY T. A. B. INOCULATION.			Twenty-five sera examined 25 days after primary T. A. B. inoculation.
		6 months.	12 months.	18 months.	
T. O. {	50	2	1	2	—
	100	10	12	11	—
	200	7	2	2	3
	300	1	—	—	5
	500	—	—	—	16
	750	—	—	—	1
	1,000	—	—	—	—
A. O. {	25	2	4	2	—
	50	8	8	12	2
	100	9	3	1	4
	200	1	—	—	15
	300	—	—	—	4
	500	—	—	—	—
B. O. {	25	—	—	—	—
	50	5	6	5	—
	100	6	7	8	2
	200	8	2	2	8
	300	1	—	—	9
	500	—	—	—	6
	750	—	—	—	—

When this table is compared with Table III, which records similar intervals in the case of those who have had more than one course of T. A. B. inoculation, a definite difference is evident. It is seen that, after primary inoculation, the titre for all the three types of agglutinins has, by the end of six months, fallen to a level which, more or less, corresponds to that found amongst uninoculated persons (*vide* Table I). Such, however, is not the case with those who have had multiple inoculations, as seen from a comparison of the average titres:—

Average titre six months after inoculation.			Primary inoculation.	Subsequent inoculation.
T. O.	..	..	140	410
A. O.	..	..	78	260
B. O.	..	..	138	460

experiments on laboratory animals, that the injection of a large dose of antigen may be followed by a well-marked negative phase, advantage was taken to test if this was the explanation of the above coincidence. At the same time the effect of two doses of T. A. B. vaccine—0.5 c.c. and 1.0 c.c.—on the elaboration of 'O' agglutinins was determined separately, since it occurred to us from the data collected by Ledingham and Schütze (1931) that after secondary and subsequent responses the antibody-forming mechanism may get so sensitized that a small stimulus, like the first dose of T. A. B. vaccine, may lead to the production of a large enough amount of 'O' antibody thus making the second injection unnecessary.

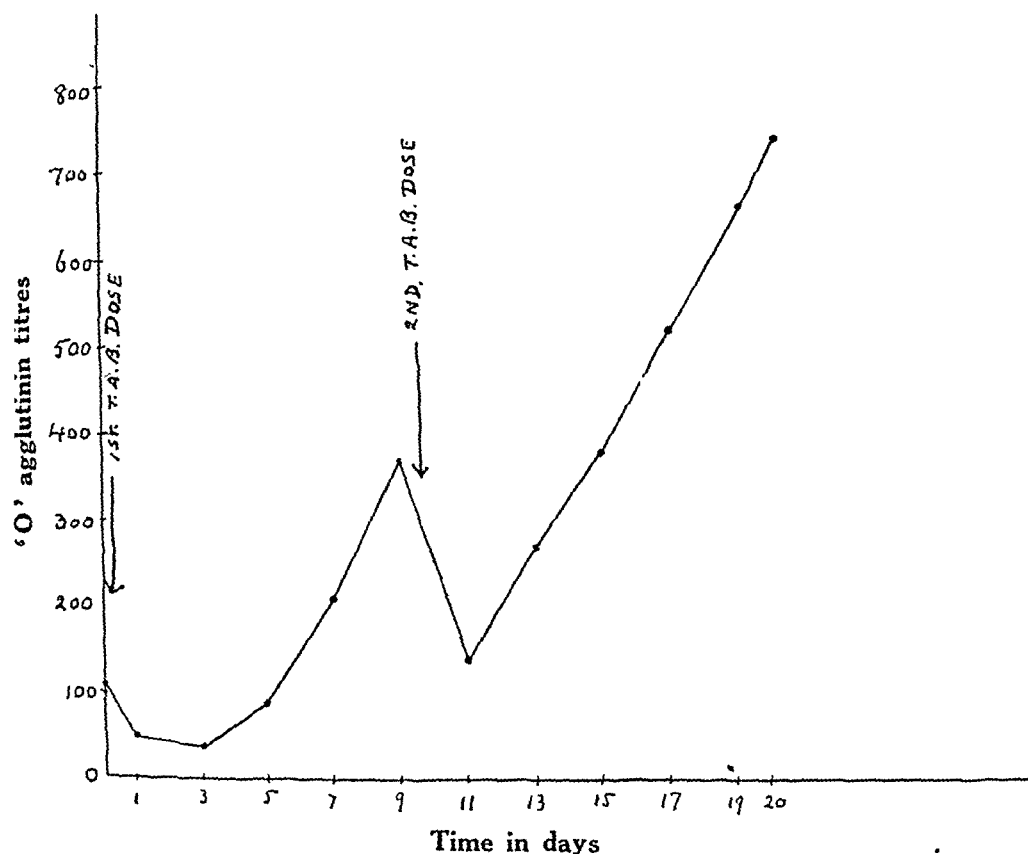


Fig. 2. Agglutinin production: response of previously inoculated persons to T. A. B. inoculation.

Individuals for this experiment were, therefore, selected from those who had had more than five courses of T. A. B. inoculation. They were bled before the commencement of the new course and every second day till ten days after the second dose. Their average titres for T. O. agglutinins are charted in Fig. 2.

It is noticed that each injection results in a temporary decrease in the titre of 'O' antibody. The induction or the negative phase lasts about four to five days

obtained from a number of individuals which in no case was less than 25. At the same time, the average titres at the end of 12 and 18 months are included for comparison. Both the titres and the time are plotted on an arithmetical scale in preference to a logarithmic scale as with the latter small differences at the bottom of the scale are disproportionately enhanced in graphic representation, whilst large differences at the summit of the curve are minimized.

It is seen from this figure that, in a person who has been inoculated before, the curve of 'O' agglutinin titre tends not only to rise higher than in one who has not been inoculated before, but also to remain high over a longer period.

The fall of agglutinins in the two types of individuals is compared in Table VII where the residual agglutinins are represented in percentages of the highest average titre resulting from T. A. B. inoculation:—

TABLE VII.

*The fall of agglutinins (a) after primary inoculation
and (b) after subsequent inoculation.*

Period after T. A. B. inoculation in months.	RESIDUAL AGGLUTININS AFTER T. A. B. INOCULATION IN INDIVIDUALS.	
	Inoculated primarily, per cent.	Inoculated previously, per cent.
3	71	90
6	32	66
12	27	28
18	24	20

It will be seen that while the titres correspond at the end of 12 months, the maximum fall in the case of those having their first inoculation takes place within the first six months. In the previously inoculated individual, on the other hand, the fall is gradual and a fairly high titre is maintained for about 12 months. From the end of the year onwards, the titre in both types of individuals runs, more or less, parallel on a level which corresponds with that found in persons who have never been inoculated.

*The 'negative phase' and the effect of two doses on the production of
'O' agglutinins.*

Cases of enteric group of fevers have lately come to notice where the natural infection followed soon after the T. A. B. inoculation in as high a proportion as 10 per cent of the total number inoculated. In view of what is known from

experiments on laboratory animals, that the injection of a large dose of antigen may be followed by a well-marked negative phase, advantage was taken to test if this was the explanation of the above coincidence. At the same time the effect of two doses of T. A. B. vaccine—0.5 c.c. and 1.0 c.c.—on the elaboration of 'O' agglutinins was determined separately, since it occurred to us from the data collected by Ledingham and Schütze (1931) that after secondary and subsequent responses the antibody-forming mechanism may get so sensitized that a small stimulus, like the first dose of T. A. B. vaccine, may lead to the production of a large enough amount of 'O' antibody thus making the second injection unnecessary.

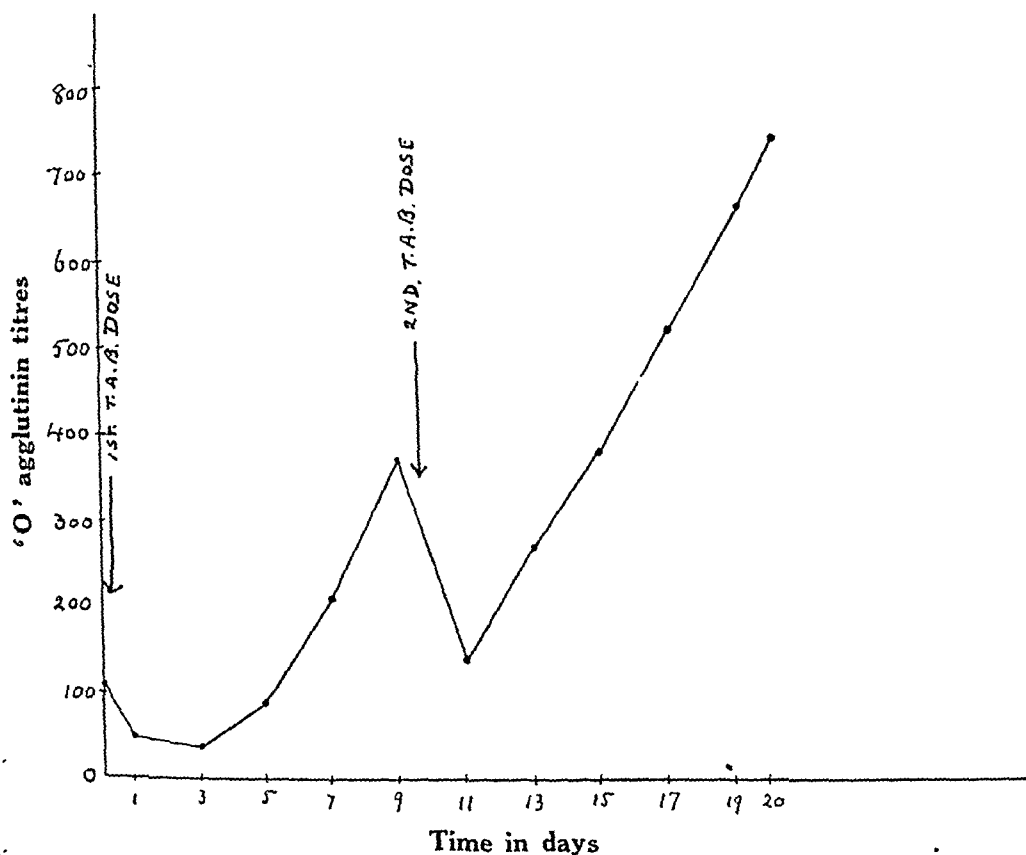


Fig. 2. Agglutinin production: response of previously inoculated persons to T. A. B. inoculation.

Individuals for this experiment were, therefore, selected from those who had had more than five courses of T. A. B. inoculation. They were bled before the commencement of the new course and every second day till ten days after the second dose. Their average titres for T. O. agglutinins are charted in Fig. 2.

It is noticed that each injection results in a temporary decrease in the titre of 'O' antibody. The induction or the negative phase lasts about four to five days

both with the first as well as with the second dose, this being the time taken by the titre to reach the same level as that before the inoculation. In no case was a prolonged negative phase noticed, although, as stated before, in 10 to 15 per cent of individuals examined the highest level of 'O' titre did not rise higher than what is met with amongst uninoculated individuals. A summation of the agglutinin producing effect by two doses is seen from Fig. 2 to take place, the second dose raising the titre to a height which is more than double of what resulted from the first dose. It is thus shown that repeated T. A. B. inoculations do not lead to a change in the reactivity of the antibody-forming apparatus such as results when infection takes place with a living virus; nor does a negative phase play any rôle in this type of immunization.

The immunological significance of 'O' agglutinins.

Having studied the manner in which 'O' agglutinins are elaborated in response to T. A. B. inoculation, it was next considered desirable to determine if they afforded any protection in terms of two well-known immunity reactions, namely, phagocytosis and passive immunization. For comparison, sera from a rabbit immunized with identical doses of T. A. B. vaccine and from a typhoid patient were included. Although the 'O' antibody content of the three sera differed it was so adjusted by dilution that the final concentration of this antibody was the same in all the sera tested. The presence of 'Vi' antibody was excluded in as low a dilution as 1 : 5, whilst the bearing of 'H' agglutinins on protection tests, such as is attributed by Springut (1927) to this antibody, was annulled by the fact that its titre, more or less, corresponded in the three types of sera.

A strain of intermediate type (Felix, Bhatnagar and Pitt, *loc. cit.*) of *B. typhosus* (Ty Razmak A) was utilized for these tests. The results of experiments are summarized in Table VIII:—

TABLE VIII.

Phagocytosis and passive immunization with 'O' antibody of animal and human origin.

Sera.	Titre of 'O' agglutinins.	PHAGOCYTOSIS OF STRAIN TY RAZMAK A.		PASSIVE IMMUNIZATION OF MICE.	
		Number of organisms phagocyted by 100 leucocytes.	Cells taking part, per cent.	Dose of serum in c.c.	Protected against
T. A. B. rabbit ..	500	110	56	0.25	4 M. L. D.
T. A. B. human ..	500	148	70	0.25	6 M. L. D.
Typhoid patient .	500	230	98	0.25	10 M. L. D.

Note.— 1 M. L. D. = 200×10^6 organisms of strain Ty Razmak A.

This table demonstrates that all the sera with an 'O' antibody content of 500 agglutinins opsonize and protect to a significant degree. A striking discrepancy, well marked in both the immunity reactions, is, however, shown by the three types of sera. The 'O' antibody resulting from a natural infection (typhoid patient's serum) is seen to be far superior both in its phagocytosis promoting action as well as in passive protection, when compared to the same antibody elaborated in response to T. A. B. inoculation. Even with the latter a difference is evident, the 'O' antibody of human origin recording better results than one produced in a rabbit with identical procedure.

These results indicate that inherent qualitative differences exist in the 'O' antibody produced by various immunizing processes. The immunity conferred by a living bacterial cell (typhoid patient's serum), measured in terms of 'O' antibody, cannot be approached by artificial immunization with a killed antigen, although the agglutinin titres may be identical in the two cases. Similarly, the slight superiority of 'O' antibody of T. A. B. human serum may be due to the fact that this antibody is not a pure product of immunization since it contains a large component which comes from natural sources. It is contended that these experiments offer an explanation of the lack of parallelism between antibody titre and the power of protection, observed by many workers, where the source of origin of the immune body was not taken into consideration.

DISCUSSION.

The results of the experiments on the sera of over 500 persons, presented in this paper, have a direct bearing on the theory and practice of T. A. B. inoculation. In conformity with other observers (Giglioli, *loc. cit.*; Dulaney *et al.*, *loc. cit.*; Dennis and Berberian, *loc. cit.*) it has been shown that inoculation with T. A. B. vaccine results in the production of a considerable amount of 'O' antibody, the immunological significance of which, and that of the smooth 'O' antigen that gives rise to it, has been well established in prophylaxis by Felix (*loc. cit.*), Felix and Olitzki (*loc. cit.*), Arkwright (*loc. cit.*), Ibrahim and Schütze (1928), Topley (*loc. cit.*), and others.

It is observed that 'O' agglutinin production by *B. paratyphosus* A falls significantly short as compared with the other two organisms included in a T. A. B. vaccine. A similar statement is recorded by Perry and Bensted (1929). From a study of its agglutinogenic activity in laboratory animals, Bhatnagar, Freeman and Gera (*loc. cit.*) concluded that a low antigenic response was the inherent property of the smooth 'O' antigen of this organism since it was noticed when immunization was carried out with live, heated and phenolized suspensions administered by either the intravenous or subcutaneous route.

A fundamental difference between primary and secondary response to T. A. B. inoculation in human subjects has been demonstrated. With a primary stimulus, the maximum rise of 'O' antibody is much less than that obtained with secondary and subsequent stimuli. Besides, the fall of titre is much quicker in the former case, so much so, that at the end of six months the 'O' antibody titre corresponds to that met with amongst uninoculated individuals (*vide* Tables I and VI, and Fig. 1). This difference finds its explanation in the fact that the first T. A. B. inoculation

confers an increased power of response to any identical subsequent stimulation. As it is generally accepted that the maintenance of immunity in the general population is due in some degree to repeated antigenic stimuli given by sub-clinical doses of natural infection, this observation is all the more interesting, since the individuals experimented on had ample opportunities of being exposed to enteric infection in their early life, so that the primary stimulation of the antibody-forming apparatus in this direction did not commence with the first administration of T. A. B. vaccine.

Support for our view is forthcoming (a) from similar experience in laboratory animals (Glenny and Sudmersen, 1921 ; Glenny, 1925 ; Topley, 1933) and (b) from a statistical analysis of epidemic data of enteric group of fevers amongst British troops in India (Report on the Health of the Army, War Office, London, 1933). Experience in the army in India shows definitely that there is a higher incidence of enteric during the first year of service of British troops in the country than in later years. This fact may be in a considerable degree due to the practice of repeating T. A. B. inoculation annually with the result that an added amount of immunity is obtained later. There is of course the possibility, mentioned above, that in Indian surroundings the antigenic stimulus suggested may play a part, this representing what might almost be called 'acclimatization'.

Before 1929 the population under consideration was given a single 1 c.c dose of T. A. B. vaccine every 12 months. Since then, two doses—0.5 c.c. and 1.0 c.c.—are administered every 18 months. Our observations on 'O' agglutinin titres at different periods after inoculation, primary or secondary, very strongly suggest that the interval between a first inoculation and re-inoculation is longer than is advisable.

The inclusion of an effective 'Vi' fraction of *B. typhosus* in the T. A. B. vaccine would be a step in the right direction so that a vaccine of maximum immunizing value might be produced. It was first shown by Felix and Pitt (1934), by experiments on mice, that this new antigen shares its place with the smooth 'O' antigen in virulence and in protective action. These observations have since been confirmed by the study of pathogenesis of typhoid fever in man (Kaufmann, 1935; Felix, Krikorian and Reitler, *loc. cit.*). Our experiments, however, indicate that when a heat-killed and carbolized vaccine prepared from a 'Vi' strain is employed, the human body fails to elaborate the 'Vi' antibody even to as low a titre as 1:10 (Table IV). The explanation of this anomaly is to be found in the harmful effect on the 'Vi' antigen of exposure to the action of phenol pointed out by Felix and Bhatnagar (*loc. cit.*). Stuart and Krikorian (*loc. cit.*) have similarly drawn attention to some deleterious effect of phenol on the 'O' antigen of *B. typhosus*. Taken in conjunction with these two statements, the facts recorded in this communication present a strong case for renewed efforts in search of a preservative which will leave the immunogenic value of the antigenic components of T. A. B. vaccine unimpaired.

Apart from the immediate bearing these observations have on the special problem of T. A. B. inoculation, they are of a more general immunological interest as well. Felix and Bhatnagar (*loc. cit.*) referred to a phenomenon of 'functional deficiency' in the case of the 'Vi' antibody. They established that this antibody resulting from immunization with formolized extracts of virulent cultures of

B. typhosus showed a well-marked discrepancy between its agglutinin titre and its protective power, when compared to the same antibody formed in response to immunization with the live organisms. The experiments summarized in Table VIII show a striking difference of a similar nature in the behaviour of 'O' antibody produced against a killed antigen and that developed in response to a natural infection. As many weighty inferences are drawn in immunological investigations from the presence of agglutinins, it is suggested that the lack of relationship between the antibody titre and the power of protection, so far as it relates to those antibodies which are of value in immunity, noted by many observers, may find an important explanation in the physical and chemical processes to which an antigen is subjected before being utilized as an immunizing agent.

SUMMARY.

(1) T. A. B. inoculation leads to the production of a considerable amount of 'O' antibody. In the case of *B. paratyphosus* A this response is not evident to the same extent as that for the other two organisms.

(2) A fundamental difference exists between primary and secondary response to T. A. B. inoculation in human beings. With a primary stimulus the maximum rise of 'O' antibody titre falls significantly short of that obtained with secondary and subsequent stimuli. It is, therefore, suggested that the second T. A. B. inoculation should be practised at the end of six months.

(3) In those individuals who have had multiple inoculations, the 'O' agglutinin titre remains fairly high for 12 months only. The desirability of repeating T. A. B. inoculation every year is, therefore, stressed.

(4) In spite of the inclusion of a 'Vi' strain of *B. typhosus*, the present T. A. B. vaccine does not produce any 'Vi' antibody. The explanation of this anomaly is given.

(5) Qualitative differences in 'O' antibody resulting from various immunizing processes are demonstrated. An explanation of the lack of relationship between the titre of this antibody and its behaviour in immunity reactions is advanced.

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THE AGGLUTINOGENIC AND AGGLUTINATING ACTIVITIES OF *B. PARATYPHOSUS* A.

BY

CAPTAIN S. S. BHATNAGAR, M.D., PH.D., M.R.C.P., I.M.S.,

ASSISTANT SURGEON J. F. FREEMAN, D.T.M., I.M.D.,

AND

JEMADAR G. C. GERA, I.M.D.

(From the District Laboratory, Razmak.)

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IN another communication (Bhatnagar, Freeman and Dhilon, 1937), while studying the nature of immunity resulting from T. A. B. inoculation in human beings, it was found that the production of 'O' agglutinins against *B. paratyphosus* A was significantly lower as compared with those for the other two organisms. On account of the practical importance of this observation, the agglutinogenicity and the agglutinability of this member of the typhoid-paratyphoid group were made the subject of a short study.

Three strains of *B. paratyphosus* A were selected for this purpose, viz., (i) *Mears* which is incorporated by many laboratories in various parts of the world in their T. A. B. vaccine, and (ii) and (iii) two other strains (*ASA* and *RK*) recently isolated by this laboratory from human cases.

Rabbits were immunized with these strains intravenously, the suspensions used being (a) live, (b) heated at 53°C. for one hour, (c) heated at 53°C. for one hour and then carbolized (0.5 per cent), and (d) steamed for one hour at 93°C., this being the temperature at which water boils in Razmak (height 6,556 feet above sea-level). The total dosage in each case was $1,200 \times 10^6$ organisms divided into three doses of 200, 400, and 600 million organisms administered at two-day intervals. The animals were bled on the sixth day after the last injection.

The immune sera thus obtained were tested against a strain of *B. paratyphosus* A (*OAK*) particularly sensitive to 'O' agglutination and supplied to us by the courtesy of Lieut.-Colonel R. F. Bridges, R.A.M.C., Officer-in-charge, Enteric Laboratory, Kasauli. The technique of agglutination followed was that described by Felix and his co-workers.

In the experiments to be described, 'H' agglutination was not estimated as it is recognized that the flagellar 'H' antigens and the antibodies that react with them play no important part in infection and in immunity.

THE PRODUCTION OF 'O' AGGLUTININ ON IMMUNIZATION WITH
DIFFERENT VACCINES OF *B. paratyphosus A.*

TABLE I.

*A comparison of 'O' antibody production by B. paratyphosus A,
B. typhosus and B. paratyphosus B.*

Number of rabbit.	IMMUNIZATION OF RABBITS WITH VARIOUS STRAINS.			TITRE OF 'O' ANTI-BODY.	
	Type of organism.	Strain.	Dose in millions of organisms.	Injected intra-venously.	Injected subcutaneously.
41	<i>B. paratyphosus A.</i>	Live .. {	<i>Mears</i> 1,200	3,000	1,000
46			<i>ASA</i> 1,200	5,000	..
47			<i>RK</i> 1,200	5,000	..
42		Heated at 53°C. for one hour. {	<i>Mears</i> 1,200	1,000	500
49			<i>ASA</i> 1,200	2,000	..
51			<i>RK</i> 1,200	3,000	..
43		Phenolized .. {	<i>Mears</i> 1,200	1,000	500
50			<i>ASA</i> 1,200	2,000	..
53			<i>RK</i> 1,200	3,000	..
44		Heated at 93°C. for one hour. {	<i>Mears</i> 1,200	100	50
54			<i>ASA</i> 1,200	150	..
52			<i>RK</i> 1,200	200	..
57	<i>B. typhosus</i>	Ty Razmak, live .. {	1,200	20,000	10,000
58			1,200	10,000	7,500
59			1,200	10,000	10,000
60			1,200	5,000	5,000
61	<i>S. ærtrycke</i>	OB heated at 93°C. for one hour.	1,200	5,000	2,000

Note.—The figures present the redenominators of titres, i.e., the highest dilution of serum in which a partial (+) agglutination was observed by the naked eye according to the scheme described under Table II.

Table I summarizes the 'O' agglutination results obtained with three different strains of *B. paratyphosus A.* For comparison, results with a strain of *B. typhosus* (Ty Razmak A) and of *B. paratyphosus B* (OB, Schütze, 1930) are included, against which sera were prepared by identical procedure.

It is recognized that experiments on the small number of animals used will not be fully convincing but the constant results in successive tests make it possible to draw certain conclusions. It is seen that none of the strains of *B. paratyphosus A.*, whether injected live, killed, or phenolized, lead to the elaboration of 'O' antibody to the same degree as either *B. typhosus* or *B. paratyphosus B* similarly treated.

This is in accord with our previous finding (Bhatnagar, Freeman and Dhilon, *loc. cit.*) where, in an examination of over 500 sera of human subjects after T. A. B. inoculation, a similar divergence in the content of 'O' antibody was observed, titres against *B. typhosus* and *B. paratyphosus* B being much higher than against *B. paratyphosus* A.

The three strains of *B. paratyphosus* A, between themselves, show a marked difference in the production of 'O' antibody. The sera obtained with the *Mears* strain, whether used in the live state, heated, or carbolized, give a lower titre as compared with the other two strains. Whilst the highest titre obtained with *Mears* strain, when used in the live state for immunization, was 1 : 3,000, that with the other two strains was 1 : 5,000—a difference quite significant when the low agglutinogenic capacity of this organism is taken into consideration. When heated and carbolized suspensions were employed a similar discrepancy was found to exist.

It was carefully ascertained that all the strains were used in as smooth a state as possible by daily sub-culturing and by colony selection; in fact the colonies of strain *Mears* presented at all times a more smooth appearance than the other two strains and were completely stable in salt concentrations up to 6.8 per cent.

In order to determine whether the route of animal inoculation had any influence on low 'O' antibody production, the *Mears* strain was injected subcutaneously in identical doses and the antibody production compared with that obtained with strains of *B. typhosus* and *B. paratyphosus* B. The results are included in Table I and are found to be of the same order.

So far as these tests go they suggest that the *Mears* strain for use in vaccine is probably of lower antigenic value than other more recently isolated strains.

THE HEAT STABILITY OF SOMATIC 'O' ANTIGEN OF *B. paratyphosus* A.

The results incorporated in Table I go to show that when all the three strains of *B. paratyphosus* A, employed in these experiments, are exposed to a temperature of 93°C. for one hour, their agglutinogenic capacity is almost entirely lost, the titres obtained with a dose of $1,200 \times 10^6$ organisms being 1 : 100 (*Mears*), 1 : 150 (*ASA*) and 1 : 200 (*RK*). This observation is of interest in view of the fact that [it] is generally accepted that the somatic antigens are highly heat stable. A definite difference in this respect is shown between *B. paratyphosus* A and the *B. typhosus* and *B. paratyphosus* B strains examined.

THE PRESENCE OF 'Vi' ANTIGEN IN CERTAIN STRAINS OF *B. paratyphosus* A.

Following the differences, noted above, in the agglutinogenic power of strain *Mears* on the one hand and the strains *ASA* and *RK* on the other, it was considered desirable to investigate whether Indian strains of *B. paratyphosus* A contained an antigen of the nature of the 'Vi' antigen of *B. typhosus*. During the course of these investigations attention was drawn to a paper by Felix and Pitt (1936) in which

they have demonstrated the presence of 'Vi' antigen in various *Salmonella* types by the use of acid treated bacteria. Felix, Krikorian and Reitler (1935) had previously expressed their inability to detect this antigen both in the freshly isolated strains and in stock cultures of paratyphoid strains by the same methods which led Felix and his co-workers to discover it in certain strains of *B. typhosus*.

As a routine all freshly isolated strains of *B. paratyphosus A* were examined on the lines adopted for demonstration of 'Vi' antigen in the case of *B. typhosus*. Strains of stock cultures were also included for comparison. Whilst the latter, including the strain *Mears*, at no time showed any evidence of the presence of 'Vi' antigen, one of the freshly isolated strains—*ASA*—gave indications of its presence. Its agglutination reactions against a pure 'O' serum are presented in Table II:—

TABLE II.

Agglutination by pure 'O' serum.

		AGGLUTINATION OF STRAINS OF <i>B. PARATYPHOSUS A.</i>					
Serum.	Dilution.	<i>ASA</i> GROWN ON LEMCO AGAR.				Mears.	Schott-müller.
		2ND DAY AFTER ISOLA-TION.		9th day.	16th day.		
		Living.	Heated at 60°C. for one hour.				
Pure 'O' serum.	100	+±	++±	++±	+++	+++	+++
	200	+	++±	+±	++±	++±	++±
	500	Tr.	++	+	++	++	++
	1,000	F. tr.	+±	Tr.	+±	+±	++
	2,000	—	+	—	+	+±	+±
	5,000	—	±	—	±	+	+
Virulence to mice ..		$\frac{1}{12}$	..		$\frac{1}{12}$	..	
Dose in millions of organisms.		100	..	..	500	..	..

Note.—The interpretation of agglutination is according to the following scheme:—

Observed with naked eye.	+++	Plentiful sediment, supernatant fluid clear.
	++	Plentiful sediment, supernatant fluid turbid.
	+	Small sediment, flakes in supernatant fluid.
Observed with 10 × lens.	±	Very small flakes in supernatant fluid.
	Tr.	Trace.
	F. tr.	Faint trace.

It will be seen that this organism possessed a well-marked resistance to 'O' agglutination on the second day after isolation as compared with two stock strains (Mears and Schottmuller). By the 16th day it had, however, become as agglutinable as the latter two organisms or its emulsion heated to 60°C. for one hour on the second day after isolation. That this phenomenon was not due to serum fastness or any related factor became evident by an interesting decrease in its virulence side by side with the loss of inagglutinability. The results of animal experiments, included in Table II, go to show that, while on the second day after isolation a dose of 100×10^6 organisms killed 10 out of 12 mice, on the 16th day, during which time daily sub-culturing on lemco agar was resorted to, as many as 500×10^6 organisms were required to kill 8 out of 12 mice experimented on.

The loss of inagglutinability and the consequent decrease in virulence are believed to be due to the unsuitability of ordinary laboratory media—lemco agar or fresh beef or horse-flesh agar—for the maintenance of an antigen which is far more labile than the 'H' antigen itself. Various other media, such as Douglas' trypticized heart-digest agar, have so far proved disappointing in our hands. Attention is, however, drawn to the ascitic agar prepared according to the method described by Kaufmann (1935), which is giving satisfactory results. We have not tried the peptone-salt agar medium recommended by Horgan (1936) very recently.

The same experience has been met with in the case of *B. typhosus* of known 'Vi' antigen content especially when it is grown on lemco agar or fresh beef agar, where the quality of beef is poor. Two strains of this organism from fatal cases, one isolated by us (S. B.) and the other (W.) obtained through the courtesy of the Professor of Pathology, King Edward Medical College, Lahore, proved more virulent than the most inagglutinable strain so far experimented on (Ty 2, Weil and Felix, 1920), but on cultivation on lemco agar both of these strains reverted to the intermediate type (Felix, Bhatnagar and Pitt, 1934) in as short a period as seven days. This may be one of the explanations why virulence tests applied to the freshly isolated strains of *B. typhosus* prove them to be of moderate virulence only. In any experiments, therefore, the medium on which the organisms are cultivated may be found to influence the retention of 'Vi' characters and the original status of a strain as of 'Vi' type may easily be lost.

THE AGGLUTINABILITY OF *B. paratyphosus* A.

Since the adverse effect of heat on the antigenic activity of *B. paratyphosus* A was noticed, it was considered desirable to determine whether the agglutinability of this organism suffered *pari passu* under similar circumstances. The agglutination of various strains after heating at 60°C., 70°C., and 93°C. for one hour was, therefore, tested against a pure 'O' serum. The results obtained with one of these strains namely OAK, an organism very sensitive to 'O' type of agglutination, are embodied in Table III. For comparison, agglutination against the living organism is also included.

A study of Table III and Table I taken together will indicate that the effect of heat on agglutinogenic capacity and agglutinability differs. While the power to induce antibody formation is almost lost with all the three strains experimented on,

when the organisms are heated to 93°C. for one hour, agglutinability on the other hand, though reduced, is quite considerable. In addition, it is seen from Table III that the live organisms are decidedly more sensitive to agglutination as compared with the same strain heated at various temperatures.

TABLE III.

The agglutinability of B. paratyphosus A.

Serum.	Dilution.	THE EFFECT OF HEAT ON AGGLUTINATION OF OAK STRAIN OF <i>B. paratyphosus A.</i>			
		Live.	Heated at 60°C.	Heated at 70°C.	Heated at 93°C.
No. 47	100	+++	+++	+++	++
	200	+++	++	++	++
	500	+++	++	+	+
	1,000	++	++	±	Tr.
	2,000	++	+	Tr.	—
	5,000	+	±	—	—
	10,000	—	—	—	—
No. 41	100	+++	+++	++	++
	200	+++	++	++	++
	500	++	++	+	+
	1,000	++	+	±	±
	2,000	+	±	Tr.	F. tr.
	5,000	±	—	—	—
	10,000	—	—	—	—

Note.—(1) Organisms heated for one hour.

(2) Readings taken after 24 hours; two hours at 37°C., then room temperature for 22 hours.

For interpretation see note under Table II.

DISCUSSION.

The determination of the serological characters of strains of organisms incorporated in a T. A. B. vaccine is of first importance and has been especially emphasized by the studies of Grinnell (1932), and Perry, Findlay and Bensted (1933a, b;

1934a, b). Felix and his co-workers (Felix and Pitt, 1934; Felix, Bhatnagar and Pitt, 1934; Felix and Bhatnagar, 1935) have demonstrated the different serological complexes of different strains of the same organism. The practical application of these investigations is reflected in the replacement of standard laboratory strains, such as, *Rawling's* of *B. typhosus*, formerly used by many laboratories and which may have altered in antigenic constitution, by more recent strains of determined antigenic type for the purpose of vaccine manufacture.

All these studies have, however, been confined to *B. typhosus*. The paratyphoid strains forming part of the T. A. B. vaccine have not so far received investigation. The accumulated data of past few years' experience pointed to the fact that the practice of T. A. B. inoculation in a population under control did not help to reduce the incidence of paratyphoid A infection to the same degree as that of typhoid fever.

The investigation, as detailed above, shows that the *Mears* strain of *B. paratyphosus* A, used in the vaccine in a perfectly smooth state, fails to elaborate the 'O' antibody to the same degree as two other recently isolated strains included for comparison. Since the immunological significance of 'O' antibody has been well established in prophylaxis (for references see Topley, 1933), and the immunity is now recognized to depend partly on the presence of antibodies *per se* and partly on accompanying cellular changes which are intimately connected with antibody formation itself, this study on experimental animals, taken in conjunction with a similar experience in human beings (Bhatnagar, Freeman and Dhillon, *loc. cit.*), would strongly stress the desirability of enhancing the immunizing value of the present paratyphoid A component of the T. A. B. vaccine by the substitution of a recently isolated strain, of suitable serological complex, as practised for *B. typhosus*, in place of the older *Mears* strain.*

A divergent effect of the thermic influence on the property of agglutinability and that of agglutininogenicity has been demonstrated in this communication. That the biological properties of a bacterial antigen may be affected differently by physical and chemical agents has been the experience of other observers also. Thus, Eisenberg and Volk (1902), while determining the nature of the union of antigen and antibody, concluded that heat and other agencies destroyed the agglutinophore and left the haptophore groups relatively unaltered. Felix and Pitt (*loc. cit.*) state that the agglutininogenic properties of both the 'Vi' and the 'O' antigen of *B. typhosus* are not impaired by treatment with alcohol, while the agglutinability by the 'Vi' antibody of alcohol-treated suspensions is almost annulled.

The loss of resistance to 'O' agglutination and the accompanying decrease in virulence of freshly isolated highly virulent strains, when grown on ordinary laboratory media, suggest the possibility that a labile factor of the same nature as the 'Vi' antigen of *B. typhosus* is responsible for this change. As any S→R variation was carefully guarded against, this study shows that the mere presence of smooth 'O' antigen does not connote the highest degree of virulence in paratyphoid A strains. Those organisms which possess this new factor should, therefore, be included in the preparation of a T. A. B. vaccine and optimum conditions aimed at for its maintenance.

* We have been informed that, on the results of a comparison of strains, this procedure has been adopted by the Central Research Institute, Kasauli, in the preparation of their T. A. B. vaccine.

624 *Agglutinogenic and Agglutinating Activities of B. paratyphosus A.*

SUMMARY.

(1) The production of 'O' antibody by *B. paratyphosus A* falls significantly short in comparison with *B. typhosus* and *B. paratyphosus B* when immunization is carried out with live, heated or phenolized suspensions and by either the intravenous or the subcutaneous route.

(2) The *Mears* strain of *B. paratyphosus A* when compared with two recently isolated strains produces a much lower antigenic response as judged by the agglutinin titre.

(3) Certain strains of *B. paratyphosus A* are resistant to 'O' agglutination and are of high virulence. Their inclusion in a T. A. B. vaccine is advocated.

(4) Exposure to 93°C. for one hour almost annuls the agglutinogenic activity of *B. paratyphosus A*. Its agglutinability at this temperature, however, remains relatively unaltered.

(5) The unsuitability of ordinary laboratory media for the maintenance of high virulence in the typhoid-paratyphoid group of organisms is demonstrated.

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ADSORPTION OF ANTIGENS BY ANTIBODIES OR VICE VERSA.

Part III.

THE EFFECT OF ELECTROLYTES ON THE RATE OF FLOCCULATION OF TOXIN-ANTITOXIN MIXTURES OF DIPHTHERIA AND TETANUS.

BY

B. N. GHOSH, D.Sc.

(University College of Science and Technology, Calcutta),

AND

N. N. RAY, M.Sc.

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SINCE Ramon's (1922*a* and *b*) observation that, of a series of mixtures of diphtheria toxin and antitoxin, the neutral or the balanced mixture flocculates more quickly than the others, a large number of papers on this flocculation reaction have been published. The influence of the nature of the toxin and the antitoxin on the rate of flocculation has been studied by Glenney *et al.* (1925). It has been found that the rate depends markedly on the potency of the toxin. As a rule, the more potent the toxin, the quicker was the flocculation obtained with a particular antitoxin. Again, using a given toxin the time of flocculation varied with different samples of antitoxin.

The reaction of the medium has also an important effect on the rate of flocculation. Schmidt (1930*a* and *b*) observed that it (the rate of flocculation) does not alter appreciably between the range of pH 5.5 and pH 8.0. At pH 4.49 and pH 9.5 the rate is very slow, while at pH 10.0 flocculation does not occur. The effect of salts on the rate of flocculation has been found to depend on their concentration. In low concentrations they usually favour the flocculation, while at high concentrations they retard it. Thus, Schmidt (1930*b*) records that in high concentrations the anions ClO_4 , SCN , ClO_3 , NO_3 , Br , IO_3 , SO_4 , Cl , NO_2 inhibit flocculation, and arranging them in order of their inhibiting power he obtained the following series $\text{ClO}_4 > \text{SCN} > \text{ClO}_3 > \text{NO}_3 > \text{Br} > \text{IO}_3 > \text{SO}_4 > \text{Cl} > \text{NO}_2$.

Since the process of peptization is the reverse of that of flocculation it is to be expected that high concentration of salts will favour the peptization of the floccules.

Marrack and Smith (1930) noticed that diphtheria-antitoxin floccules were peptized by high concentration of salts containing the anions salicylate, iodide, thiocyanate, bromide and nitrate. The order of their peptizing power has been found to be salicylate > I > SCN > Br > NO₃.

It is to be noted, however, that although unconcentrated diphtheria antitoxin when mixed with the right quantity of the toxin flocculates easily, yet the concentrated antitoxin freed from serum albumin and the so-called euglobulin fraction does not flocculate on mixing with the toxin (*cf.* Ramon, 1922b). This failure of flocculation might lead one to suppose that concentrated diphtheria antitoxin does not combine with the toxin under ordinary laboratory conditions. Eagle (1935) has, however, brought forward evidence which indicates that purified diphtheria antitoxin does combine with the toxin *in vitro*. An analysis of these facts leads to the conclusion that the diphtheria toxin-antitoxin reaction occurs in at least two stages.

The first stage consists in the adsorption of the particles of toxin by those of the antitoxin leading to the formation of neutral particles containing both toxin and antitoxin. These neutral particles we may term the 'complex primary particles', as they serve as units in the subsequent process of flocculation. The second stage consists in the aggregation of the 'complex primary particles' (formed in the first stage of reaction) into bigger ones, which finally form floccules. Thus the failure to obtain Ramon's flocculation test in purified diphtheria antitoxin solution is to be attributed to the fact that in such solution the aggregation of the 'complex primary particles' is prevented by some inhibiting factors. The present work was undertaken with a view to find out how far by a suitable choice of electrolytes and toxin, and by the adjustment of the dilution of the toxin and the antitoxin, it is possible to overcome the effect of the inhibiting factors. The results so far obtained are recorded in this paper.

EXPERIMENT.

Determination of flocculating power of a few electrolytes and non-electrolytes.

In the first place the effect of a number of substances on the rate of flocculation of mixtures of diphtheria toxin and (unconcentrated) antitoxin was studied. This enabled us to select a few electrolytes of high flocculating power and also gave us an idea of the optimum concentrations in which they should be employed in subsequent experiments.

To determine the flocculating power of a substance, a stock solution of it was prepared. The toxin and the antitoxin were suitably diluted with this stock solution in separate flasks. A fixed quantity of the diluted toxin (2 c.c.) was delivered into a series of glass tubes of uniform diameters. Different quantities of the diluted antitoxin were then added to the tubes and their volumes adjusted to a definite amount by adding the requisite quantities of the stock solution. The tubes were shaken and placed in a bath maintained at 50°C. and the time required for the first appearance of visible flocculation noted. For each series of experiments all the factors except the nature of the flocculating agent were maintained,

as far as possible, constant so that the results are fairly comparable among themselves. Some of the data obtained are recorded in Table I :—

TABLE I.

Concentration of the diluted toxin—1/5th of the original toxin.
Concentration of the diluted antitoxin—1/30th of the original antitoxin.

K_f = the time of commencement of flocculation.

Substance used.	Molar concentration.	K_f in minutes.
Glucose	0·33	Infinite.
Urea	0·17	"
Sodium chloride	0·15	180
Sodium oxalate	0·08	120
Sodium phosphate (Na_2HPO_4)	0·07	120
Sodium citrate	0·044	90
Sodium citrate	0·132	45

The data recorded in the above table show that taking sodium chloride as the standard for comparison solutions of urea and glucose inhibit flocculation, while sodium citrate has the highest flocculating power.

If the four electrolytes are arranged in the order of their flocculating power then the following series is obtained :—

Citrate > phosphate > oxalate > chloride.

It was, therefore, decided to try the effect of citrate and phosphate solutions of different concentrations on mixtures of diphtheria toxin and its concentrated antitoxin.

Preparation of concentrated diphtheria antitoxin and study of its flocculation reaction.

The major part of the diphtheria antitoxin contained in immunized horse serum is precipitated with the pseudoglobulin fraction.

The antitoxic serum was first diluted with water and then sufficient quantity of saturated ammonium sulphate solution is added to it so as to make the solution 30 per cent saturated with respect to ammonium sulphate. This precipitates the euglobulin. It was filtered and to the filtrate equal volume of 70 per cent saturated ammonium sulphate added. This makes the whole solution half saturated with respect to ammonium sulphate. The precipitate consisting of the pseudoglobulin fraction is separated by filtration and dialysed in a cellophane bag against water in a cold chamber. When it is free from ammonium sulphate it is diluted to five times its original volume with distilled water and its pH adjusted to 5·6 and the requisite quantity of tricresol added. After keeping in a cold chamber for about a week, it was filtered through a Seitz filter and used for our experiments. The remaining experimental procedure was the

same as described in the previous section. The results are recorded in Table II. From these data it will be noticed that using sodium citrate and sodium phosphate as flocculating agents concentrated diphtheria antitoxin gives flocculation with its antigen.

TABLE II.

Concentration of the diluted toxin—1/5th of the original toxin.
Concentration of the diluted antitoxin—1/30th of the original antitoxin.

Electrolyte used.	Molar concentration.	Time of commencement of flocculation in minutes.
Sodium citrate {	0.06	120
	0.12	90
	0.17	45
Sodium phosphate (Na_2HPO_4) .. {	0.14	100
	0.21	80

The effect of the potency of the toxin on the rate of flocculation was also tried. The results are to be found in Table III. These data show that with a given electrolyte and a given sample of concentrated antitoxin the rate of flocculation depends markedly on the potency of the toxin. In fact if the potency of the toxin is less than 7 L_f units per c.c. there is hardly any flocculation within a reasonable period of time.

TABLE III.

Strength of the diluted toxin—1/5th of the original toxin.
Strength of the diluted antitoxin—1/20th of the original antitoxin.
Strength of the sodium citrate solution—3.5 per cent.

Number of concentrated antitoxin sample.	Number of toxin sample.	Potency of toxin in L_f units per c.c.	K_f in minutes.
1 {	A	30	45
	B	16.5	120
	C	7.2	190
2 {	A	30	50
	B	16.5	128
	C	7.2	260

The agreement between the potency of the concentrated antitoxin (diphtheria) determined by the flocculation test and that determined by animal experiment.

Having observed that purified diphtheria antitoxin gives flocculation test with its antigen in presence of sodium citrate or phosphate, we tried to determine how far this test can be used as a means of measuring the potency of the antitoxin. For this purpose, simultaneous flocculation tests and animal experiments were carried out using always the same sample of toxin. This toxin sample was standardized with reference to a standard antitoxin shortly before this series of experiments were started.

The results are recorded in Table IV. It will be noticed that the agreement between the results obtained by the two methods is fair.

TABLE IV.

Flocculation of mixtures of concentrated tetanus antitoxin with its antigen.

Sample number of antitoxin.	Units per c.c. of concentrated antitoxin found by flocculation test.	Units per c.c. of concentrated antitoxin found by animal experiment.
1	960	1,000
2	1,225	1,200
3	1,650	1,600
4	1,680	1,800
5	1,445	1,500
6	1,750	1,800
7	2,080	2,000
8	1,700	1,800
9	1,200	1,200

Flocculation of concentrated tetanus antitoxin by tetanus toxin.

In some preliminary experiments with a mixture of fresh antitoxic serum and tetanus toxin, we obtained flocculation in presence of 3.5 per cent citrate solution. Frequently, however, two zones of flocculation at two different toxin-antitoxin ratios were obtained.

Animal experiments showed that only one of these zones corresponds with the balanced mixture of tetanus toxin and antitoxin; the other zone is non-specific. It was thought that purification and concentration of the antitoxin might eliminate the non-specific reaction as it was comparatively weak and gives only the specific one. Some experiments were, therefore, made with concentrated tetanus antitoxin. The concentration was effected in exactly the same way in which diphtheria antitoxic serum was concentrated. The results of flocculation test along with those of animal tests are recorded in Table V. The potency of the antitoxin is expressed in the table in American units. It will be noticed that there is fair agreement between the two sets of data. It is needless to add that these results are as yet of a preliminary nature but they appear to be of sufficient significance to be incorporated in this paper. Further work in this line is in progress.

TABLE V.

Strength of the solution—3·5 per cent.

Sample number of antitoxin.	Units per c.c. obtained by flocculation test.	Units per c.c. obtained by animal test.
1	1,550	1,500
2	1,800	2,000
3	1,280	1,400
4	1,050	950
5	1,450	1,600
6	1,650	1,800
7	2,166	2,250
8	1,790	1,850

It may be mentioned that in none of the above experiments the non-specific reaction was noticeable.

SUMMARY.

1. The effect of a few substances on the rate of flocculation of diphtheria toxin-antitoxin mixture has been determined. The order of flocculating power of the sodium salts of electrolytes used was citrate > phosphate > oxalate > chloride.

Urea and glucose solutions do not produce flocculation under the conditions of the experiment.

2. In presence of sodium citrate or disodium hydrogen phosphate flocculation is obtained with purified and concentrated diphtheria antitoxin. The rate of flocculation has been found to depend on the potency of the toxin and on the concentration of the electrolytes used.

3. In presence of citrate of sodium purified and concentrated tetanus antitoxin also gives flocculation. The data so far obtained show that the balanced mixture flocculates most quickly compared with the other mixtures.

In conclusion, we thank very warmly the authorities of the Bengal Immunity for offering excellent facilities for carrying out this piece of work.

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CANCER IN INDIA.

BY

VISHWA NATH, M.A. (Harvard), M.D. (Pb.), F.R.C.P.I., D.P.H.,
D.T.M. & H. (Cantab.),

AND

KHEM SINGH GREWAL, M.B., B.S. (Pb.), PH.D. (Cantab.).
(*From the King Edward Medical College, Lahore.*)

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OUR last paper on Cancer in India, published in this *Journal* in July 1935, embodied results of a survey covering the provinces of the Punjab, Delhi, United Provinces, and Bihar & Orissa. This paper presents data since collected from Madras and Burma.

A much larger number of autopsy records were available for examination in this survey (6,416) than was the case in Northern India (1,234) where popular prejudice greatly reduces the number of post-mortem examinations by pathologists. The records under review dealt with post-mortem examinations carried out by pathologists to the Medical College, Rangoon, and the Medical College, Madras. The latter included 404 examinations carried out at the Raipurum Medical School, Madras.

Of the 6,416 post-mortem examinations made, a diagnosis of malignant disease was arrived at in 260, showing an incidence of 1 in 24.7. Of the 2,454 examinations made in Rangoon, malignant disease was detected in 97 and of the 3,962 carried out in Madras 163 showed presence of malignant disease. This gives an incidence of 1 in 25.3 for Rangoon and 1 in 24.3 for Madras. It must not be inferred from this that the incidence of cancer as judged from autopsy records is practically the same amongst Burmans and Madraseds. Rangoon has a mixed population. This mixture is reflected in the record of 2,454 post-mortem examinations under discussion. One thousand five hundred and ninety-four of these examinations were carried out on Indian subjects and 860 on Burmans. The incidence of malignant disease in the former group was 1 in 39 and in the latter 1 in 16.

The proportion of carcinoma to sarcoma in the Rangoon figures was 3·5 : 1 and in Madras 6·85 : 1 ; with the Rangoon and Madras figures taken together the proportion is 5 : 1.

The site of maximum incidence is the gastro-intestinal tract, taken together with liver and pancreas. In Rangoon this site accounts for 50 per cent of the cases—the incidence amongst Indians and Burmans being practically the same. In Madras, the figure is even higher (62·1 per cent). The combined figure is 58 per cent. In Madras the site second in order of frequency is buccal (14·6 per cent) and cancer of the female genital tract (5·1 per cent) comes third. In Rangoon, cancer of the female genital tract (24·3 per cent) comes second and buccal cancer (9·5 per cent) occupies the third place.

Two hundred and sixty deaths from malignant disease, of which 205 were due to cancer, is too small a figure to use for purposes of comparison. Table II, indicating proportional frequency of cancer as affecting different sites in the human body, is therefore of very limited significance as compared with the English data quoted.

The incidence of cancer was also ascertained from records of medico-legal autopsies performed in Rangoon from 1930 to 1934. Records of 3,150 examinations were studied relating to deaths from violence, poisoning, or accidents. Malignant disease was discovered in 17 cases in this series. It was not the cause of death but discovery incidental to the investigation made. Of the 17, sixteen were cases of cancer and one of sarcoma—a male. Of the cases of cancer 12 were male and 4 female. The order of frequency according to sites affected was gastro-intestinal tract including the liver (six cases), buccal (four cases), female genital tract (two cases), and miscellaneous (four cases).

Records of morbid histology show that of a series of 11,801 specimens examined in Madras 2,236 were diagnosed malignant and that in Rangoon of 2,814 specimens examined a diagnosis of malignant disease was returned in 741. In other words one out of every five specimens in Madras and one out of four in Rangoon were diagnosed to be histologically malignant.

A study of the regional incidence based on the records of Madras and Rangoon taken together (Table VII) shows that cancer of the female genital tract (19·7 per cent) represents the site of maximum incidence. Buccal cancer (18·5 per cent) comes next and that cancer of the breast and skin both compete for the third place (13 per cent). Cancer of the penis comes next in order of frequency (10·2 per cent) and then the gastro-intestinal tract and liver (5·2 per cent).

Viewing this incidence separately in Madras and Rangoon the order of frequency is found to be the same in either town for the first two sites, viz., female genital tract and buccal. The third place, however, is occupied in Madras by cancer of the skin and the fourth by cancer of the breast. In Rangoon it is vice versa.

Considering the distribution of regional incidence for the two sexes (Tables VIII and IX) we find that buccal cancer shows a disproportionately high incidence in men (27·3 per cent) as compared with women (10·3 per cent). The disproportion is evident to nearly the same extent when the figures for Madras and Rangoon are considered separately. The communal distribution of this form of cancer shows its highest incidence amongst Mohammedan males both in Rangoon (50 per cent)

and Madras (48·9 per cent). In Rangoon, Hindu males show an incidence of 39·5 per cent and Burmese males of 20·3 per cent. The figures of incidence of buccal cancer—much lower for women—show the same relative communal distribution.

Incidence of cancer of the gastro-intestinal tract including the liver falls heavier on the male (7·2 per cent) than on the female (2·6 per cent). This disproportion is evident both in the Madras and Rangoon figures. Communally the incidence is a little higher amongst Hindu males in Madras and Mohammedan males in Rangoon.

So far as cancer of the skin is concerned, the incidence is again higher in the male (16·5 per cent) and lower in the female (10·4 per cent). Rangoon and Madras figures also bear out this observation separately. Communally both Mohammedan males and females show higher incidence of this form of cancer, and in Rangoon the non-Indian population, particularly male, more than the Indian.

Cancer of the breast showed a higher incidence in Rangoon (27·8 per cent) than in Madras (25·4 per cent). Its incidence in Rangoon was nearly twice as high amongst non-Indian women as amongst Indian.

Cancer of the female genital tract—mostly carcinoma of the cervix—showed (Table IXa) its highest incidence amongst Hindu women in Rangoon (47·2 per cent), Burmese women came next (41·7 per cent); Mohammedan women showed 33·3 per cent. In Madras too the highest incidence was amongst Hindu women (42·1 per cent) and lowest amongst Mohammedans (26·7 per cent).

As regards penile cancer (Table VIII), this is practically confined to the non-circumcized communities. The Rangoon figures show an incidence of 14·8 per cent, Madras of 22·4 per cent. In Rangoon the highest incidence was amongst the Burmese with 24·7 per cent, Hindus came next with 11·8 per cent. In Madras figures for Hindus were highest with 24·5 per cent, for other non-Mohammedan communities 18·5 per cent.

Clinical records of cases treated in the in-patients' departments of teaching hospitals in Madras and Rangoon showed (Table X) that out of a total of 222,442 patients treated 5,776 were diagnosed to be suffering from malignant disease. Out of a total of 163,470 in-patients Madras showed 4,922 cases of malignant disease and Rangoon out of 58,972 in-patients 854.

In the Rangoon General Hospital the incidence worked out to be 1 in 69; in the Madras General Hospital 1 in 25; in the Madras Maternity Hospital 1 in 28; in the Raipurum Medical School Hospital 1 in 89.

The age of highest incidence of cancer according to records of autopsies, morbid histology, and patients treated indoors in the Rangoon and Madras hospitals (Tables III, V and XII) is between 40 and 50. The incidence is fairly high even between 30 and 40. Sarcomata shows high incidence between 20 and 40, although noticeable frequency begins right from infancy.

The records show (Table XVII) that, in Madras, buccal cancer shows the highest incidence (37·9 per cent). Cancer of the female genital tract comes next (31·08 per cent) and cancer of the gastro-intestinal tract and liver occupies the third place. In Rangoon cancer of the female genital tract (30·7 per cent) occupies the first place, gastro-intestinal tract (21·7 per cent) the second and buccal cancer (13·9 per cent) comes third. There is not much difference between the percentage

incidence for breast in Madras (7.25 per cent) and Rangoon (8.8 per cent) or of penile cancer—Madras 5.45 per cent, Rangoon 5.1 per cent. These figures represent incidence on the two sexes taken together.

Viewing percentage incidence separately for the two sexes (Table XVI) one finds that the high incidence of buccal cancer in Madras becomes remarkably high for the male (60.15 per cent) against female (17.9 per cent). Same is true of Rangoon (Table XVIb)—male 22.9 per cent, female 5.6 per cent (Table XVIc). Cancer of the gastro-intestinal tract also shows heavier incidence on the male. In Madras the incidence was 15.55 per cent for the male and 4.3 per cent for the female. In Rangoon it was 36.1 per cent for the male and 8.45 per cent for the female. Similarly is the case with cancer of the skin; in Madras 2.95 per cent for the male and 0.9 per cent for the female; in Rangoon 5.4 per cent for the male and 0.7 per cent for the female.

Considering the same incidence by communities one finds (Table XVI) that buccal cancer shows the highest incidence in the Mohammedan male both in Madras (70 per cent) and Rangoon (Table XVIb) (37.8 per cent). In Rangoon, Hindu males come next with 35.5 per cent and Burmese last with 13 per cent. Incidence amongst women is in the same communal order.

As regards cancer of the gastro-intestinal tract the Rangoon figures again give (Table XVIb) the Mohammedan male (39.1 per cent) a lead over the Hindu (31.3 per cent) and Burmese (36.5 per cent) males. Males of communities other than these three showed an even higher incidence (41.3 per cent). The total incidence over this group was, however, so small that much importance cannot be given to this percentage incidence. Burmese women showed this form of cancer in a higher percentage than women of other communities, but here again the total number dealt with was small.

Cancer of the skin showed (Table XVIb) a higher incidence on the Burmese (9.9 per cent) in Rangoon than on the Hindus (3.6 per cent).

Practically the entire incidence of penile cancer was on the uncircumcized (Tables XVI and XVIb). Taking cancer incidence on the male, penile cancer represents 10.8 per cent of the total in Rangoon and 10.55 per cent in Madras. In Rangoon the Burmese lead with 17.7 per cent, Hindus come next with 7.6 per cent and other non-Mohammedan males with 4.8 per cent. In Madras the Hindus show 12.5 per cent and other non-Mohammedan males 5.1 per cent.

Cancer of the female genital tract (Table XVIa and c) has a practically similar incidence in Rangoon (58.5 per cent) and Madras (60.9 per cent). The preponderating incidence is on Hindu women both in Rangoon (70.6 per cent) and Madras (62.3 per cent); Burmese women in Rangoon with 57.5 per cent come next.

Cancer of the breast shows (Table XVIa and c) a higher incidence in Rangoon (16.9 per cent) than in Madras (12.05 per cent) and in the former town higher in Burmese women (20 per cent) than amongst Hindu (4.4 per cent).

The ratio of sarcoma to carcinoma in the Rangoon General Hospital was 1 : 7; in the Madras General Hospital 1 : 8.4; in the Raipurum Medical School, Madras, 1 : 6.6.

Table XIX shows the number of cases of malignant disease returned by provincial hospitals with indoor accommodation. Our investigation convinced us

that these returns were not as accurate as they could be. In Burma, the proportion of cases of malignant disease to total number of in-patients treated was 1 : 164 and in Madras 1 : 89.

DISCUSSION.

The foregoing data and those set forth in our previous paper (Nath and Grewal, 1935) offer only a very rough measure of the incidence of cancer in India. In the absence of proper vital statistics, one has to seek indices of prevalence from sources such as we have utilized. Out of a thousand deaths from all causes in England, cancer accounted for 132 in the year 1934 (Ministry of Health, 1934). In the 6,416 pathological examinations considered in this paper cancer accounts for 40.5 deaths per thousand. If mortality, minus deaths from infectious diseases which do not prevail in England but which raise our death rate much above the English level, were considered, the proportion of cancer deaths will not be found to differ much from that obtaining in England or other Western countries.

In England the crude death rate from cancer per million persons living was 1,563 in 1934 (Ministry of Health, *loc. cit.*). We have no data for offering such accurate information, but a consideration of the incidence of cancer among 3,150 persons who met with sudden death through violence, poisoning, or accident and who but for such an end could constitute a random sample of the living population may prove interesting. If cancer prevailed in the general population at the same rate as in this group (which of course it cannot, since the group was not diluted by subjects of insusceptible age), incidence of cancer would be 3.4 times as high as in England. This is an extremely crude, perhaps ridiculous, comparison but it is offered as giving some support to our general impression that the incidence of malignant disease in the East is not far behind that in the West.

Both the pathological and medico-legal autopsy records show that the commonest site of cancer is the gastro-intestinal tract taken together with the liver and pancreas. This was also the conclusion offered by autopsy material reviewed in our last paper (Nath and Grewal, *loc. cit.*) and represents the site of maximum incidence particularly in the male almost all over the world.

The incidence was much higher in Madras (63.4 per cent) than in Rangoon (50 per cent). According to clinical records of all the provinces surveyed the prevalence of buccal cancer stands highest in Madras and there is no doubt that the same is true of peptic ulcer. Whatever the rights of the ulcer-cancer controversy regarding the stomach may be from an academic point of view, epidemiologically the association of the highest peptic ulcer rate with that of the highest incidence of the cancer of the gastro-intestinal tract and also of buccal cancer in one and the same province cannot be devoid of significance. The preponderating incidence of all three conditions is on the male sex.

Buccal cancer is a lesion of an exposed site and consequently not difficult of diagnosis. The incidence figures from all types of records, therefore, support one another and confirm buccal as a site of high frequency. The same is true of cancer of the cervix—which represents, so far as cancer in women is concerned, the site of maximum incidence with us as well as most other countries. Same was the case in England until quite recently, but birth control and scientific care of parturition and higher level of personal hygiene have now given cancer of this site a position subordinate to that of cancer of the breast.

As a site of maximum incidence of cancer the position of the gastro-intestinal tract, which is not an easily accessible site for purposes of diagnosis, rests, however, on the indubitable evidence of post-mortem examinations. That the histology and clinical records do not assign it a position of importance is a fact of no consequence. Scarcity of material for biopsies from such inaccessible sites and errors in diagnosis arising from the supposed rarity of cancer in India would explain the comparatively smaller figures of incidence of cancer of the gastro-intestinal tract under these heads.

The Indian population in Rangoon is drawn mostly from the Madras Presidency. Ethnologically, therefore, the people amongst whom the incidence of cancer was surveyed in this paper were Dravidian or Burmese—different to the people in Northern India, the locus of the last survey. It seems common social usages, personal habits and hygiene, more than race, determine a uniformity of incidence of cancer. For example, the incidence of cancer of the female genital tract—mainly the cervix—falls heaviest and at an earlier age period on the Hindu women in Madras as well as Rangoon, just as it did in Northern India—Burmese women come next and Mohammedan last. Early marriage, the trauma of frequent confinements, attendant focal sepsis, and, in the majority, chronic malnutrition must have an ætiological bearing on the frequency of cancer of this site amongst Hindu women.

Similarly, buccal cancer which showed the highest incidence amongst Mohammedan males in Northern India shows high incidence in Madras and Rangoon. Lack of oral hygiene, chewing *pan* reinforced with lime and tobacco and retention of the quid over long periods causing chronic irritation and abrasion of the buccal mucosa must be responsible for this relatively higher incidence. The following table from page 98 of the Chief Medical Officer's report for 1934 (Ministry of Health, *loc. cit.*) will afford interesting comparison :—

International nomenclature number.	Site.	MALE.		FEMALE.	
		Total deaths from cancer.	Deaths per thousand.	Total deaths from cancer.	Deaths per thousand.
45	Buccal cancer ..	2,812	98	537	16
46	Cancer of gastro- intestinal tract, liver and pan- creas.	17,704	614	15,958	489
	Uterus ..	..	..	4,313	132
	Penis ..	156	5	..	..
	Skin ..	618	21	500	15
	Breast ..	55	2	6,551	200

In Rangoon, cancer of the breast, skin, and penis showed relatively higher incidence in the Burmese. Benign papillomata of the penis are of relatively frequent occurrence in Rangoon. This may be due to the rigidity of standards prevailing in that centre in the matter of diagnosis of penile cancer. In addition to the usual histological criteria of malignancy, the presence of metastases and clinical signs are also ascertained; a diagnosis based on a constellation of all these features will be incontrovertible, but some early cases escape detection under these circumstances. The difficulty of labelling cancer of the penis on evidence other than histological will be illustrated by the following quotation from Willis (1935): 'Leighton (1932) stated that in carcinoma of the penis the inguinal glands are affected early but Kuttner (1900) and Morson (1930) found that the early enlargement of these glands was inflammatory only and that metastasis was long delayed and relatively infrequent; 32 per cent according to Kuttner. This author has shown also that the initial metastasis from penile cancer may occur in the pelvic lymph glands, while the inguinal glands remain unaffected. According to Bamey (1907) penile cancer yields metastasis in 60 per cent of cases but these appear usually late'.

Whatever the nature and importance of a common general factor possibly governing the causation of cancer may be, one cannot under-estimate the significance of the obvious and controllable carcinogenic factor of irritation as operating on particular sites like the mouth, the cervix uteri, the skin, and probably the gastrointestinal tract.

Dr. Stevenson studied the incidence of cancer in England by dividing the population into five classes on the basis of economic and educational standing and has brought out the significant fact that, so far as cancer of the exposed sites is concerned, its incidence in the most highly placed group Class I is nearly two-fifths of that of Class V (Annual Report of the Registrar-General, 1921).

It would be difficult in any country to bring the economic level of the population at large to Class I level but it is possible through education and public health propaganda to mitigate the evil effects of social customs and personal habits which promote the incidence of cancer.

The collection of the foregoing data was made possible only through the kind help of the superintendents and pathologists of the teaching hospitals visited and we express our grateful thanks to them.

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TABLE I.

Incidence of malignant disease as shown in the autopsy records of the following institutions.

Name of institution.	Total number of autopsies performed.	Total number diagnosed to have malignant disease.	Terratoma.	Carcinoma.	Sarcoma.	Endothelioma.	Hodgkin's disease.	Ratio of sarcoma to carcinoma.	Ratio of malignant disease to total number of autopsies.
Medical College, Rangoon.	2,454	97	1	74	21	1	..	1 : 3.5	1 : 25
Medical College, Madras.	3,558	154	1	131	18	2	2	1 : 7.3	1 : 23
Raipuran Medical School, Madras.	404	9	..	6	2	1	..	1 : 3	1 : 45
Total for above-mentioned institutions.	6,416	260	2	211	41	4	2	1 : 5.1	1 : 24.7
Medico-legal post-mortems, Rangoon.	3,150	17	..	16	1	..	..	1 : 16	1 : 185; 5.3 per 1,000 post-mortems.

TABLE II.

Incidence of malignant disease and regional distribution of cancer according to communities as ascertained from post-mortem records from 1909-35 of the pathological department of the Medical College, Madras.

Name of community.	CANCER.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.	Total number of autopsies.	Ratio of malignant disease to total number of autopsies.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Hard.	Soft.					
Hindu males ..	..	1	..	71	1	1	14	..	98	1	10	11	..	2	2,388	1 : 22
Hindu females ..	6	..	2	5	6	1	..	..	20	..	2	2	..	..	621	1 : 31
Mohammedan males ..	..	..	..	1	1	..	..	..	2	..	1	1	..	..	82	1 : 27
Mohammedan females ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	12	..
Other males ..	..	..	..	4	1	..	1	..	6	..	3	3	..	..	288	1 : 32
Other females ..	1	..	1	2	1	..	1	1	7	1	..	1	1	..	71	1 : 8
Unknown ..	..	..	..	3	1	..	..	..	4	..	..	..	..	..	96	1 : 16
TOTALS ..	7	1	3	86	11	2	16	1	137	2	16	18	1	2	3,558	1 : 25

TABLE IIa.

Incidence of malignant disease and regional distribution of cancer according to religion and sex as ascertained from post-mortem records from 1926-35 of the pathological department of the Medical College, Rangoon.

Religion and sex.	CANCER.								SARCOMA.			Hodgkin's disease.	Teratoma.	Endothelioma.	Number of post-mortems performed.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Hard.	Soft.	Total.			
Hindu males	..	2	..	13	4	..	30	..	22	1	8	9	..	..	..
Hindu females	4	..	1	..	..	..	..	..	5	..	..	..	..	..	Indians 1,594.
Mohammedan males	..	..	..	1	..	..	..	..	1	..	..	..	..	..	Burmese 860.
Mohammedan females	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Rates of malignant disease in Indians 1: 39, in Burmese 1: 16.
Burmese males	..	..	..	17	1	..	5	..	23	1	7	8	1	..	..
Burmese females	14	..	..	4	1	..	1	..	20	..	3	3	..	..	..
Other males	..	..	..	2	1	..	..	..	3	..	1	1	..	..	..
Other females	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
TOTALS ..	18	5	1	37	7	..	36	..	74	2	19	21	1	1	..

TABLE III.

Incidence of malignant disease according to age as determined from post-mortem records of the Medical College, Rangoon, Medical College, Madras, and Raipurum Medical School, Madras.

Age.	CARCINOMA.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Hard.	Soft.	Total.		
1-5	..	..	..	..	..	..	..	..	..	..	..	..	..	..
6-10	..	..	..	1	..	..	..	..	1	..	..	..	..	1
11-15	..	..	..	2	..	..	..	..	4	..	..	..	..	..
16-20	..	..	..	8	1	..	1	..	14	2	6	2	..	..
21-25	4	2	..	11	1	1	3	..	20	1	8	9	..	1
26-30	5	2	1	14	3	1	6	..	29	1	4	5	1	..
31-35	5	1	..	19	5	..	1	..	30	..	7	7	..	..
36-40	5	1	..	20	8	..	4	..	38	..	3	4	2	..
41-45	3	..	3	9	1	..	3	..	14	..	1	3	..	..
46-50	1	..	..	3	2	..	2	..	15	..	..	1	..	..
51-55	..	..	..	3	..	..	2	..	6	..	..	..	..	..
56-60	..	..	..	..	..	..	1	..	1	..	..	..	..	..
61-65	..	..	..	..	..	..	2	..	..	..	..	..	..	..
66-70	..	..	..	..	..	..	..	..	..	..	..	..	..	..
71-75	..	..	..	..	..	..	..	..	..	..	..	..	..	..
76-80	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Above 80	..	..	..	6	1	..	..	..	7	..	..	..	..	..
Age not known ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
TOTALS ..	25	3	4	124	27	2	25	1	211	4	37	41	4	2

TABLE IV.

Incidence of malignant disease as based on morbid histology records of the following institutions.

Name of institution.	CARCINOMA.							SARCOMA.			Total section.					
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Hard.		Soft.	Total.	Endothelioma.	Teratoma.	Hodgkin's disease.
Medical College, Rangoon.	122	41	86	21	114	51	127	11	573	29	77	106	20	25	17	2,814
Medical College, Madras.	277	123	144	75	221	180	243	8	1,271	78	249	327	94	55	17	11,801
Raipurum Medical School, Madras.	30	58	54	17	68	53	42	5	327	28	91	119	15	9	2	..
TOTALS ..	429	222	284	113	403	284	412	24	2,171	135	417	552	129	89	36	..

TABLE V.

Showing distribution of carcinoma according to age and site and of sarcoma according to age alone as gathered from pathological histology records of the Medical Colleges, Rangoon and Madras. and Medical School, Raipurum.

Age.	CARCINOMA.								Sarcoma.	Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.			
1-5 ..	..	..	..	..	..	..	6	..	9	18	4	1
6-10 ..	..	..	..	..	..	..	1	..	2	25	6	5
11-15 ..	..	..	..	..	1	3	12	..	18	21	4	3
16-20 ..	..	..	..	2	2	6	12	..	38	39	13	4
21-25 ..	29	7	4	2	18	10	24	..	94	42	14	5
26-30 ..	47	19	26	14	36	24	44	..	211	70	10	3
31-35 ..	56	23	21	13	48	21	35	..	217	50	20	1
36-40 ..	65	23	51	17	65	38	58	..	322	71	14	2
41-45 ..	61	23	26	9	42	30	32	..	253	47	8	3
46-50 ..	68	34	54	16	44	41	41	..	299	45	9	3
51-55 ..	24	12	34	12	23	16	28	4	153	27	7	0
56-60 ..	20	27	32	9	49	35	37	5	214	31	7	..
61-65 ..	9	10	9	2	13	6	12	..	61	10	2	..
66-70 ..	3	7	7	2	7	11	10	5	52	3	1	..
71-75 ..	1	3	..	..	3	4	1	1	13	2	..	..
76-80 ..	..	3	0	2	2	2	1	..	10	..	..	..
81 and above ..	..	..	1	..	..	..	..	..	1	..	..	..
Unknown ..	33	24	17	13	50	37	55	5	233	51	10	2
TOTALS ..	429	222	284	113	403	284	412	24	2,171	552	129	36

TABLE VI.

Showing distribution of malignant disease according to sex, religion, and site from pathological histology records availed of during the survey.

Religion and sex.	CARCINOMA.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Skin.	Buccal.	Miscellaneous.	Rodent ulcer.	TOTAL.	Soft.	Hard.	TOTAL.		
Hindu males ..	0	171	9	61	128	189	177	1	736	191	74	265	45	17
Hindu females ..	263	0	152	17	51	69	66	2	620	111	18	129	39	2
Mohammedan males	0	1	0	4	11	41	23	3	83	21	10	31	5	1
Mohammedan females	18	0	14	0	9	10	13	1	65	6	2	8	4	1
Burmese males ..	..	31	3	6	18	26	40	4	128	18	6	24	7	7
Burmese females ..	81	..	56	3	12	17	24	1	194	25	5	30	5	2
Other males ..	0	15	1	8	19	36	34	7	120	15	7	22	7	5
Other females ..	49	0	42	7	11	9	10	3	131	12	5	17	9	0
Unknown ..	18	4	7	7	25	6	25	2	91	18	8	26	8	1
TOTALS ..	429	222	284	113	284	403	412	24	2,171	417	135	552	129	36

TABLE VII.

Regional distribution of carcinoma per hundred cases based on the biopsy records of the pathology laboratories of the following institutions.

Name of institution.	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.
Medical College, Rangoon ..	21.4	7.2	15.0	3.6	19.9	8.9	21.6	1.8
Medical College, Madras, and Raipurum Medical School, Madras.	19.2	11.3	12.3	5.7	18.0	14.5	17.8	0.8
Percentage incidence for the above institutions taken together.	19.7	10.2	13.0	5.2	18.5	13.0	18.9	1.1

TABLE VIII.

Regional distribution of carcinoma per hundred male cases based on the records of the pathology laboratories of the following institutions.

Name of institution.	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	..	14.8	1.8	5.0	30.5	11.8	32.8	3.2	279
Medical College, Madras, and Raipurum Medical School, Madras.	..	22.4	1.0	8.2	26.2	18.1	23.1	0.7	788
Percentage incidence for the above institutions taken together.	..	20.7	1.1	7.2	27.3	16.5	25.5	1.4	1,067

TABLE VIIIa.

Regional distribution of carcinoma per hundred Hindu male cases based on the records of the pathology laboratories of the following institutions.

Name of institution.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	11.8	2.7	5.2	39.5	9.2	31.5	..	76
Medical College, Madras, and Raimapuram Medical School, Madras.	24.5	1.06	8.6	24.1	17.9	23.2	0.15	660

TABLE VIIIb.

Regional distribution of carcinoma per hundred Mohammedan male cases based on the records of the pathology laboratories of the following institutions.

Name of institution.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	2.7	..	8.3	50.0	5.5	33.3	..	36
Medical College, Madras, and Raimapuram Medical School, Madras.	..	..	2.1	48.9	69.1	23.4	5.3	47

TABLE VIIIc.

Regional distribution of carcinoma per hundred other male cases based on the records of histology laboratories of the following institutions.

Name of institution.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	..	..	2.5	15.3	28.2	41.0	13.0	39
Medical College, Madras, and Rairupurum Medical School, Madras.	18.5	1.2	8.6	30.8	16.05	22.0	2.3	81

TABLE VIId.

Regional distribution of carcinoma per hundred Burmese male cases based on the records of the pathology laboratories of the Medical College, Rangoon.

Name of institution.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	24.2	2.4	4.7	20.3	14.0	31.2	3.1	128

TABLE IX.

Regional distribution per hundred female cases based on the records of the pathology laboratories of the following institutions.

Name of institution.	CANCER.						
	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.
Medical College, Rangoon	44.7	27.8	2.4	9.9	5.8	12.0	0.7
Medical College, Madras, and Raimun Medical School, Madras.	40.4	25.4	2.7	10.5	9.1	10.8	0.7
Percentage incidence of the above institutions taken together.	40.6	27.1	2.6	10.3	8.2	11.1	0.69
							1,010

TABLE IXa.

Regional distribution of carcinoma per hundred Hindu female cases based on the records of the pathology laboratories of the following institutions.

Name of institution.	CANCER.						
	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.
Medical College, Rangoon	47.2	13.9	..	13.9	8.3	16.6	..
Medical College, Madras, and Raimun Medical School, Madras.	42.1	25.1	2.9	10.9	8.5	10.3	0.3
							36
							584

TABLE IXb.

Regional distribution of carcinoma per hundred Mohammedan female cases based on the histology records of the following institutions.

Name of institution.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	33.3	16.6	..	25.0	8.3	16.6	..	12
Medical College, Madras, and Rairupurum Medical School, Madras.	26.7	22.6	..	13.2	15.1	20.7	1.9	53

TABLE IXc.

Regional distribution of carcinoma per hundred other female cases based on the histology records of the following institutions.

Name of institution.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	36.7	36.7	8.3	8.3	2.0	6.0	2.0	49
Medical College, Madras, and Rairupurum Medical School, Madras.	37.7	29.2	10.9	4.8	6.1	8.5	2.4	82

TABLE IXd.

Regional distribution of carcinoma per hundred Burmese female cases based on the histology records of the Medical College, Rangoon.

Name of institution.	Female genitals.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total cases.
Medical College, Rangoon ..	41.7	28.9	1.5	8.7	6.1	12.2	0.5	194

TABLE X.

Neoplastic diseases as shown in the records of the following institutions.

Number.	Name of institution.	Total number of in-patients treated.	Teratoma.	Carcinoma.	Sarcoma.	Endothelioma.	Hodgkin's disease.	Ratio of sarcoma to carcinoma.	Ratio of cases of malignant diseases to total number of in-patients treated.
1	General Hospital, Rangoon.	58,972	22	704	107	3	18	1: 7.0	1: 69
2	General Hospital, Madras.	74,827 (1930-34)	21	2,632	310	2	18	1: 8.4	1: 25
3	Maternity Hospital, Madras.	38,290 (1930-33)	1	1,375	5	..	..	1: 275.0	1: 28
4	Raipuram Medical School, Madras.	50,353 (1930-34)	6	472	71	..	9	1: 6.6	1: 89
Totals ..		222,442	50	5,183	493	5	45	1: 10.5	1: 38.5

TABLE XI.
Incidence of malignant disease as based on hospital records of the following institutions.

Name of institution.	CANCER.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Soft.	Hard.	Total.		
General Hospital, Madras.	41	185	235	351	1,538	52	207	23	2,632	172	138	310	21	18
Raipurum Medical School, Madras.	43	69	60	64	159	28	43	6	472	46	25	71	6	9
Maternity Hospital, Madras.	1,308	0	27	17	..	4	19	..	1,375	4	1	5	1	..
General Hospital, Rangoon.	110	46	65	194	120	26	135	8	704	62	45	107	22	18
Lady Dufferin Hospital, Rangoon.	166	..	14	1	4	..	10	..	195	1	..	1	1	..
TOTALS ..	1,668	300	401	627	1,821	110	414	37	5,378	285	209	494	50	45

TABLE XII.

Showing distribution of carcinoma according to age and site and of sarcoma according to age alone as gathered from hospital records of the Medical Colleges, Rangoon and Madras, and the Medical School, Raipurum.

Age.	CARCINOMA.										Sarcoma.	Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.					
1-5	..	..	1	..	2	..	2	..	5	20	1	..	..	3
6-10	..	..	..	..	4	..	3	..	7	25	1	..	..	4
11-15	..	..	1	..	5	1	4	..	12	23	2	..	1	0
16-20	..	1	4	6	12	8	11	1	50	61	2	..	..	4
21-25	..	7	10	15	44	5	15	2	147	57	2	..	..	4
26-30	..	49	31	54	113	12	31	0	429	55	3	..	..	4
31-35	..	165	30	49	179	9	36	2	999	52	6	..	1	4
36-40	..	238	40	86	407	13	57	2	569	37	7	..	..	4
41-45	..	333	61	79	244	7	45	2	726	16	1	..	..	5
46-50	..	264	47	108	334	20	57	5	926	11	1	..	..	3
51-55	..	265	84	67	173	7	40	6	532	9	1	..	..	2
56-60	..	169	50	71	194	6	49	7	524	9	1	..	..	1
61-65	..	101	45	33	77	7	14	2	196	5	1	..	..	..
66-70	..	33	16	13	50	3	13	4	105	41	..	..	..	..
71-75	..	13	7	4	14	1	8	2	41	1	..	..	..	..
76-80	..	3	4	2	6	1	..	..	13	1	..	..	..	..
80 and over	..	1	1	2	1	1	..	..	5	1	..	..	..	..
Unknown	26	15	11	38	62	9	29	2	92	31	4	..	..	5
TOTALS ..	1,668	300	401	627	1,821	110	414	37	5,378	494	50	6	..	45

TABLE XIII.

Combined table showing cases of malignant disease according to sex, religion, and site in the Province of Burma and Madras Presidency.

Religion and sex.	CANCER.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Hard.	Soft.	TOTAL.		
Hindu males ..	..	221	19	306	1,039	57	169	12	1,823	125	168	293	18	20
Hindu females ..	1,223	..	205	90	343	18	64	9	1,952	31	46	77	7	6
Mohammedan males	..	1	2	53	159	2	34	2	253	15	17	32	4	4
Mohammedan females	49	..	11	4	34	..	9	..	107	3	4	7	2	0
Burmese males ..	..	34	..	70	25	19	41	3	192	16	13	29	7	7
Burmese females ..	184	..	63	25	14	2	31	1	320	2	16	18	5	0
Other males ..	..	15	5	58	164	7	45	6	300	11	15	26	4	8
Other females ..	201	..	73	18	40	4	19	4	359	2	4	6	1	..
Unknown ..	11	29	23	3	3	1	2	..	72	4	2	6	2	0
TOTALS ..	1,668	300	401	627	1,821	110	414	37	5,378	209	285	494	50	45

TABLE XIV.

Combined table showing cases of malignant disease according to sex, religion, and site in the following institutions :—

(1) General Hospital, Madras, (2) Raipuram Medical School, Madras, and
(3) Maternity Hospital, Madras.

Religion and sex.	CANCER.								SARCOMA.			Teratoma.	Endothelioma.	Hodgkin's disease.
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Hard.	Soft.	TOTAL.		
Hindu males ..	0	212	19	269	997	53	143	12	1,705	110	153	263	16	17
Hindu females ..	1,175	0	202	85	338	17	58	9	1,884	28	42	70	7	5
Mohammedan males	0	1	2	31	138	2	21	2	197	13	10	23	2	3
Mohammedan females	37	0	11	3	27	0	6	0	84	2	2	4	1	0
Other males ..	0	12	4	32	154	7	25	3	237	8	10	18	1	2
Other females ..	173	0	61	10	40	4	14	3	305	1	3	4	0	0
Unknown ..	7	29	23	2	3	1	2	0	67	2	1	3	1	0
TOTALS ..	1,392	254	322	432	1,697	84	269	29	4,479	164	221	385	28	27

TABLE XIVa.

Combined table showing cases of malignant disease according to sex, religion, and site in the following institutions :—

(1) General Hospital, Rangoon, and (2) Lady Dufferin Hospital, Rangoon.

Religion and sex.	CANCER.							SARCOMA.							
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Soft.	Hard.	Total.	Teratoma.	Endothelioma.	Hodgkin's disease.
Hindu males ..	0	9	0	37	42	4	26	0	118	15	15	30	2	1	3
Hindu females ..	48	0	3	5	5	1	6	0	68	3	4	7	0	0	1
Mohammedan males	0	0	0	22	21	1	13	0	56	7	2	9	2	0	1
Mohammedan females	12	0	0	1	7	0	3	0	23	2	1	3	1	0	0
Burmese males ..	0	34	0	70	25	19	41	3	192	13	16	29	7	0	7
Burmese females ..	184	0	63	25	14	2	31	1	320	16	2	18	5	2	0
Other males ..	0	3	1	26	10	0	20	3	63	5	3	8	3	1	6
Other females ..	28	0	12	8	0	0	5	1	54	1	1	2	1	0	0
Unknown ..	4	0	0	1	0	0	0	0	5	0	2	2	1	0	0
Totals ..	276	46	89	195	124	26	145	8	899	63	46	108	22	4	18

TABLE XV.

Incidence of neoplastic diseases according to provinces as ascertained from the records of hospitals visited.

Name of province.	CANCER.								SARCOMA.			Endothelioma.	Hodgkin's disease.	
	Female genitals.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Hard.	Soft.			TOTAL.
Madras ..	1,392	254	322	432	1,697	84	269	29	4,479	164	222	386	27	
Burma ..	276	46	79	195	124	26	145	8	899	45	63	108	18	

TABLE XVI.

Regional distribution per hundred male cases according to communities in the Madras Presidency.

Name of community.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Hindu ..	12.5	1.1	15.7	58.5	3.1	8.4	0.7	100	1,705
Mohammedan ..	0.5	1.0	15.6	70.0	1.0	10.5	1.0	100	197
Others ..	5.1	1.7	13.1	65.0	2.95	10.5	1.3	100	237
For 100 male cases ..	10.55	1.1	15.55	60.15	2.95	8.8	0.8	100	2,139
For combined Burma and Madras.	10.5	0.9	18.9	54.0	3.3	..	..	..	..

TABLE XVIa.
Regional distribution of carcinoma per hundred females according to communities in the Madras Presidency.

Name of community.	Genitals.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Actual number of cases recorded.
Hindu ..	62.3	10.7	4.5	17.9	0.9	3.0	0.45	100	1,884
Mohammedan ..	44.0	13.1	3.5	32.1	..	7.1	0	100	84
Others ..	56.7	20.0	3.2	12.9	1.3	4.5	0	100	305
For 100 female cases	60.9	12.05	4.3	17.9	0.9	3.8	0.5	100	2,273
For combined Burma and Madras.	60.5	12.8	5.0	15.3	0.8	..	..	..	..

TABLE XVIb.
Regional distribution of carcinoma per hundred males according to communities in the Province of Burma.

Name of community.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	Total.	Actual number of cases recorded.
Hindu ..	7.6	..	31.3	35.5	3.6	22.0	..	100	118
Mohammedan ..	..	..	39.1	37.8	..	23.1	..	100	56
Burmese ..	17.7	..	36.5	13.0	9.9	21.3	1.6	100	192
Others ..	4.8	1.6	41.3	15.6	..	31.3	4.8	..	63
For 100 male cases	10.8	0.3	36.1	22.9	5.4	23.3	1.4	100	429
For combined Burma and Madras.	10.5	0.9	18.9	54.0	3.3	..	..	..	..

TABLE XVIc.

Regional distribution of carcinoma per hundred female cases according to communities in the Province of Burma.

Name of community.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Hindu ..	70.6	4.4	7.3	7.3	1.4	8.5	..	100	68
Mohammedan ..	52.1	..	4.3	30.4	..	13.0	..	100	23
Burmese ..	57.5	19.7	7.8	4.4	0.6	9.7	0.3	100	320
Others ..	51.8	22.2	14.8	..	..	9.2	1.8	100	54
For 100 female cases ..	58.5	16.9	8.45	5.6	0.7	8.5	0.4	100	465
For combined Burma and Madras.	60.5	12.8	5.0	15.3	0.8	..	..	..	..

TABLE XVII.

Regional distribution of carcinoma per hundred cases according to provinces as ascertained from the records of hospitals visited.

Name of province.	Female genitals.	Penis.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	31.08	5.45	7.25	9.65	37.9	1.9	6.0	0.64	100	4,479
Burma ..	30.7	5.1	8.8	21.7	13.9	2.9	16.1	0.89	100	899

TABLE XVIIa.

Regional distribution of carcinoma per hundred male cases according to provinces as ascertained from the records of hospitals visited.

Name of province.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	10.55	1.1	15.55	60.15	2.95	8.8	0.8	100	2,139
Burma ..	10.8	0.3	36.1	22.9	5.4	23.3	1.4	100	429

TABLE XVIIb.

Regional distribution of carcinoma per hundred Hindu male cases according to provinces as ascertained from the records of hospitals visited.

Name of province.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	12.5	1.1	15.7	58.5	3.1	8.4	0.7	100	1,705
Burma ..	7.6	..	31.3	35.5	3.6	22.0	..	100	118

TABLE XVIIc.

Regional distribution of carcinoma per hundred Mohammedan male cases according to provinces.

Name of province.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	0.5	1.0	15.6	70.0	1.0	10.5	1.0	100	197
Burma ..	..	..	39.1	37.8	..	23.1	..	100	56

TABLE XVIIId.

Regional distribution of carcinoma per hundred Burmese male cases.

Name of province.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Burma ..	17.7	..	36.5	13.0	9.9	21.3	1.6	100	192

TABLE XVII.

Regional distribution of carcinoma per hundred other male cases according to provinces.

Name of province.	Penis.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	5.1	1.7	13.1	65.0	2.95	10.5	1.3	100	237
Burma ..	4.8	1.6	41.3	15.6	0	31.3	4.8	..	63

TABLE XVIII.

Regional distribution of carcinoma per hundred female cases according to provinces as ascertained from the records of hospitals visited.

Name of province.	Female genitals.	Breast.	Gastro-intestinal tract and liver.	Buccal.	Skin.	Miscellaneous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	60.9	12.05	4.3	17.9	0.9	3.8	0.5	100	2,273
Burma ..	58.5	16.9	8.45	5.6	0.7	8.5	0.4	100	465

TABLE XVIIIa.

Regional distribution of carcinoma per hundred Hindu female cases according to provinces as ascertained from the records of hospitals visited.

Name of province.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	62.3	10.7	4.5	17.9	0.9	3.0	1.45	100	1,884
Burma ..	70.6	4.4	7.3	7.3	1.4	8.5	0	100	68

TABLE XVIIIb.

Regional distribution of carcinoma per hundred Mohammedan female cases according to provinces.

Name of province.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	44.0	13.1	3.5	32.1	..	7.1	..	100	84
Burma ..	52.1	..	4.3	30.4	0	13.0	..	100	23

TABLE XVIIIc.

Regional distribution of carcinoma per hundred Burmese female cases.

Name of province.	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Burma ..	57.5	19.7	7.8	4.4	0.6	9.7	0.3	100	320

TABLE XVIIIId.

Regional distribution of carcinoma per hundred other female cases according to provinces.

Name of province	Female genitals.	Breast.	Gastro- intestinal tract and liver.	Buccal.	Skin.	Miscellan- eous.	Rodent ulcer.	TOTAL.	Actual number of cases recorded.
Madras ..	56.7	20.0	3.2	12.9	1.3	4.5	0.9	100	305
Burma ..	51.8	22.2	14.8	..	..	9.2	1.8	100	54

TABLE XIX.

Incidence of cancer as ascertained from the records of the Inspector-General of Civil Hospitals, Burma, and from the records of the Surgeon-General, Madras.

Name of province.	Period.	Total number of in-patients treated in Class I, III and IV hospitals of the province.	Total number of cases of malignant disease treated as in-patients.	Ratio of cases of malignant disease to total number of in-patients.
Burma	1930-33 both inclusive.	432,446	2,631	1 : 164
Madras	1930-33	851,029	9,558	1 : 89

DIET SURVEYS IN SOUTH INDIAN VILLAGES.

BY

W. R. AYKROYD,

AND

B. G. KRISHNAN.

(*Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.*)

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It is often said that the diet of the Indian villager or ryot is insufficient in quantity and poor in quality. On the other hand, statements are made that the average villager, in spite of his manifest poverty, has enough 'good wholesome food' to maintain health and strength, and that the diet of the poor in towns and cities is much more deficient. The general impressions of untrained observers on such points are, however, of doubtful value, and very little exact information, collected by systematic surveys, is available about the diet of Indian peasants. Since some 80 per cent of the population live in villages, such information is essential if the problem of nutrition in India is to be defined and understood, and its collection is one of the most important objects of nutrition research.

McCay (1912) gives the composition of 'average diets' consumed by various classes of the population in Bengal, including cultivators, and also that of an 'average' Sikh diet. The dietaries of industrial workers in India have been investigated in family budget inquiries carried out by the Labour Office of the Government of Bombay (1923, 1928*a* and *b*, 1935) and by the Commerce Department of the Government of Bengal (1930); the purpose of these investigations was, however, economic, and no attempt was made to assess the physiological adequacy of the diets. Wilson, Ahmad and Mullick (1936) have recently made a detailed study of the diet of a few families in Calcutta. The Board of Economic Inquiry, Punjab (1928), has published an economic survey of a village in the Amritsar district in which some rough data about the food intake of a few families are included; it is possible that the reports of economic inquiries carried out elsewhere in the country contain similar data. Nicholls (1936) has reported the results of an investigation of the diet of 16 Ceylonese families of the labouring classes, and a similar but more detailed inquiry, extending over a year and including 15 families of peasants and 5 families of working men, has been carried out in Java (Ochese *et al.*, 1934). Surveys in adjacent countries where dietary habits are roughly similar are of interest to nutrition workers in India.

SCOPE AND METHOD OF THE PRESENT INQUIRY.

The diet of 44 families, including 274 persons, was investigated over a period of 20 days. Of these, 8 were Christian families, engaged in cultivation and coolie work, living in a small village near Chingleput in South-East Madras. Four Hindu families, distinctly more prosperous, living in a neighbouring village, were included in this survey which took place in January 1936. A larger inquiry, comprising 32 families, was organized in a number of small villages in the neighbourhood of Mayanur, about 36 miles N.-W. of Trichinopoly; this inquiry took place in July and August 1936. Twenty-nine of these were engaged in agriculture or ancillary village occupations, with some coolie work of various kinds. The occupations of the remainder were as follows: clerk in the Public Works Department; agricultural instructor at a Rural Reconstruction Centre; owner of a provision store.

The actual collection of the data was carried out, in the case of the Chingleput group, by student health visitors of the Red Cross School, Madras, and a village nurse, working under the supervision of a European lady missionary. In the Mayanur inquiry, 6 teachers attached to the Servants of India Society Rural Reconstruction Centre at Mayanur collaborated. To carry out such inquiries successfully it is essential to work in co-operation with agents on familiar terms with the families under investigation and trusted by them. At the outset we carefully explained to our collaborators what we wanted them to do, and remained with them for a few days of supervision, during which homes were visited, food weighed out, and the details filled in on an appropriate form. When we were sure that they had fully grasped the method and purpose of the inquiry, we left them to 'carry on' themselves. They visited each home twice daily at the time of the preparation of meals and weighed each item of food to be consumed on portable balances. In the Mayanur inquiry each worker visited 6 families daily; apart from the fact that the teachers concerned had other work to do, this is about the maximum number that one worker can deal with at a time, because families must be visited early at the time of preparing the food for the day. The question of making a conventional allowance for 'waste', as is usual in family dietary inquiries, scarcely arises. If food prepared on one day was not entirely consumed, it would be kept for the next and added to that day's reckoning. It is always advisable, in presenting the results of dietary inquiries, to describe the actual method of field investigation; when such information is not given, one has no means of assessing the reliability of the final conclusions. Our experience in field dietary inquiries may be of value to other investigators attempting this line of research. We have little doubt of the substantial accuracy of the food-intake data collected in these inquiries. To the best of our knowledge, these are the first detailed village-diet surveys to be carried out in India.

It may be objected that a three-weeks' inquiry is inadequate to give a picture of diet throughout the year, and that for a complete survey repeated investigations at other seasons would be desirable. In actual fact, however, the diet of very poor South Indian peasants does not vary much with season and the results of even a short survey at one season may throw light on food intake throughout the year. In the Chingleput area, the chief rainy season is in October, November,

and December, so that January follows the rains ; in the Mayanur district there had been fair rain during the month previous to the inquiry.

In the Mayanur district, 323 school children belonging to the families studied and other families of similar status were weighed and measured and examined for evidence of food deficiency disease. No examination of adults was carried out.

To show the attitude of the families to the investigation, we cannot do better than quote a passage from a letter from Mr. A. L. Subramanyan who participated actively in the Mayanur inquiry :—

‘ In the case of some families there was no difficulty even from the beginning. It was necessary to create confidence by removing their prejudice, suspicion, fear, and general apathy, all due to their poverty and illiteracy. Only the 32 families for which we are able to send figures co-operated with us to the very end. About half a dozen dropped off after a few days. In some families the male members were favourably inclined though the women protested ; in others, it was the reverse. The prejudice against weighing things to be cooked and eaten was hard to break. “ How is this weighing of our food grains going to benefit us ? Will it solve our poverty problem ? Will it help us to feed our hungry mouths ? ” were the usual remarks. Their poverty and misery, which we saw at close quarters, you may be able to see from the figures we are submitting ’.

WORKING UP OF THE DATA.

Intake of calories, proximate principles, calcium, phosphorus, and iron was calculated per consumption unit per day. To reduce families of varying age and sex composition to consumption units, the earlier International scale of family co-efficients (League of Nations Health Organization, 1932) was employed. This scale is as follows :—

Age.	CO-EFFICIENT.		
	Male.	Both sexes.	Female.
0-2 ..	..	0.2	..
2 and 3 ..	..	0.3	..
4 „ 5 ..	..	0.4	..
6 „ 7 ..	..	0.5	..
8 „ 9 ..	..	0.6	..
10 „ 11 ..	..	0.7	..
12 „ 13 ..	..	0.8	..
14 to 59 ..	1.0	..	0.8
Over 60 ..	..	0.8	.

It must be emphasized that at present we have no knowledge regarding the appropriateness of this scale for use in India. It has been employed by Nicholls (*loc. cit.*) for a similar inquiry in Ceylon, and there is no reason to suppose that any other scale in current use (e.g., Lusk's or Cathcart and Murray's) would be more satisfactory. Only by systematic investigation of the basal metabolism and food intake of both sexes at various ages can a suitable Indian scale of family consumption co-efficients be constructed.

In calculating intake of the various food factors, figures obtained in a systematic survey of the composition of Indian foods, carried out in the laboratory, were used (Ranganathan *et al.*, 1937). Analyses were made on the edible portion of the raw uncooked food.

Families have been divided into 4 groups as follows :—

GROUP I. Eight families in Overtownpet village, near Chingleput. Sixty-seven persons.

GROUP II. Four families in Karumpakkum village, near Chingleput. Twenty-eight persons.

GROUP III. Twenty-nine agricultural families in various villages in the neighbourhood of Mayanur, Trichinopoly district. One hundred and sixty-eight persons.

GROUP IV. Three families not engaged in agriculture in same district. Eleven persons.

ECONOMIC STATUS.

To arrive at a satisfactory assessment of the economic position of any group of peasant families, it would be necessary to collect data about all sources of income, indebtedness, taxes, the amount of live stock and other property owned, etc. Indian village economy is complicated, and its detailed study lies outside the scope of nutrition research. An attempt was nevertheless made, in the present investigation, to assess gross income very roughly, in terms of the value of crops produced and wages obtained for coolie work and other labour.

GROUP I was the poorest of the four. The families in this group were tenants with an average holding of 3 acres of land per household, for which rent was paid in cash and produce to the value of Rs. 3 per acre per annum. Average annual income in this group may be roughly reckoned as between Rs. 50 and Rs. 80 per family (with the exception of one more prosperous family).

GROUP II consisted of families of a distinctly more prosperous order. The total income of family 1 in this group, derived from the father's occupation as school teacher and postmaster as well as from agriculture, exceeded Rs. 1,000 per annum. In the other 3 families, annual income lay between Rs. 200 and Rs. 300.

GROUP III included families of different income and economic status, and may perhaps be regarded as a cross section of a village community. At the upper limit, we may place a Brahmin family owning 10 acres of wet land, and a considerable quantity of live stock, whose annual income may be reckoned as Rs. 300; at the lower, families supported by coolie work, leasing an acre or so of dry land, and owning one or two head of cattle, with a gross annual income under Rs. 100. Average family income in this group was somewhat higher than in Group I, being in the neighbourhood of Rs. 100 per annum. The incomes of Group IV lay between Rs. 350 and Rs. 500.

Most of the families owned some live stock including cows and she-buffaloes. During the period of investigation, however, the out-turn of milk was very small, and a number of families sold a high proportion of whatever milk was produced. Only one or two families owned sheep and goats; goat's milk was not consumed at all. Poultry were unimportant as a source of income or food.

TABLE I.

Intake of calories, proximate principles, calcium, phosphorus, and iron per consumption unit per day.

Family.	Number of persons.	Number of consumption units.	Protein (g.).	Fat (g.).	Carbo-hydrates (g.).	Calories.	Percentage of total calories derived from cereals.	Calcium (g.).	Phosphorus (g.).	Iron (mg.).
1	14	9.9	44.1	5.3	447.9	2,026	96	0.52	0.90	23.5
2	6	4.8	40.9	6.4	414.5	1,896	95	1.18	0.93	30.1
3	6	4.8	37.6	4.9	392.8	1,775	94	0.10	0.74	17.8
4	11	7.7	34.0	3.6	387.0	1,725	98	0.05	0.69	14.8
5	9	6.1	37.6	4.0	358.2	1,601	96	0.98	0.93	28.5
6	7	4.4	32.5	3.6	351.2	1,576	96	0.45	0.69	19.0
7	6	3.9	31.7	3.2	341.8	1,529	96	1.19	0.72	32.1
8	8	5.1	28.7	3.6	257.7	1,184	92	0.33	0.57	13.8
TOTAL	67	Mean 5.8	35.9	4.4	368.9	1,664	95.4	0.60	0.75	22.4
1	9	7.6	52.8	37.9	478.3	2,473	77	0.72	1.13	23.8
2	7	6.4	50.9	14.9	483.2	2,280	88	0.48	1.02	24.7
3	7	5.6	44.1	10.2	429.6	1,995	89	0.39	0.90	21.7
4	5	3.2	44.2	19.9	394.5	1,946	77	0.33	0.85	25.5
TOTAL	28	Mean 5.7	48.0	20.7	446.4	2,173	83	0.48	0.97	23.9

GROUP I.

GROUP II.

TABLE II.
Composition of the diet of various families. (Oz. per consumption unit per day).

GROUP I.			GROUP II.	
Family 1.	Family 7.	Family 3.		
Home-pounded parboiled rice .. 17.40	Home-pounded parboiled rice .. 14.90	Home-pounded parboiled rice .. 15.20		
Ragi (<i>Eleusine coracana</i>) .. 2.10	Bengal gram .. 0.94	Dhal arhar .. 1.40		
Dhal arhar (<i>Cajanus indicus</i>) .. 0.09	Beans .. 0.14	Black gram (<i>Phaseolus mungo</i>) .. 0.10		
Bengal gram (<i>Cicer arietinum</i>) .. 0.03	Tamarind .. 0.46	Bengal gram .. 0.15		
Beans .. 0.20	Coriander seeds .. 0.08	Beans .. 0.33		
Tamarind .. 0.45	Onions .. 0.03	Tamarind .. 0.90		
Coriander seeds .. 0.05	Radish .. 0.16	Coriander seeds .. 0.16		
Onions .. 0.07	Green leafy vegetables .. 0.27	Sundakai (<i>Solanum torvum</i>) .. 0.09		
Gingelly oil .. 0.01	Crabs .. 2.80	Onions .. 0.33		
Crabs .. 0.50	Fish .. 0.13	Radish .. 0.40		
Fish .. 0.50		Sorakai (<i>Lagenaria vulgaris</i>) .. 0.75		
Mutton .. 0.20		Green leafy vegetables .. 0.50		
		Brinjal (<i>Solanum melongena</i>) .. 0.09		
		Drumstick (<i>Moringa oleifera</i>) .. .		
		Coco-nut .. 0.12		
		Gingelly oil .. 0.42		
		Fish .. 0.03		
		Whole milk .. 1.10		
		Curds .. 0.48		
		Butter-milk .. 0.12		
		Sugar .. 0.09		
		Sago .. .		

TABLE II—contd.
Composition of the diet of various families. (Oz. per consumption unit per day).

GROUP III.

Family 3.	Family 7.	Family 14.	Family 22.	Family 29.
Roughly milled.. 14.80 parboiled rice.	1.70	3.00	3.00	8.28
Cholam (<i>Sorghum</i> <i>andropogon</i>), 12.20	.. 24.90	.. 17.40	.. 14.00	.. 2.79
Dhal arhar .. 2.10	.. 0.63	Cambu (<i>Pennis-</i> <i>tum typhoides</i>) 1.20	.. 2.10	.. 0.95
Cluster beans .. 0.20	.. 0.32	Dhal arhar .. 1.00	Dhal arhar .. 0.85	.. 10.20
Tamarind .. 1.10	Sweet potato 0.10	Black gram .. 0.32	Tamarind .. 0.40	.. 0.75
Amaranth leaves 0.80 (<i>Amaranthus gan-</i> <i>geticus</i>).	.. 0.35	Tamarind .. 0.46	Amaranth leaves 0.41	
Brinjal .. 3.20	Chicken .. 0.37	Coriander seeds .. 0.02		
Coco-nut .. 0.15		Onions .. 0.17		
Gingelly oil .. 0.50		Amaranth leaves 0.70		
Fish .. 0.03		Brinjal .. 0.10		
Chicken .. 0.80		Plantains .. 1.40		
Jaggery .. 0.60		Gingelly oil .. 0.36		

TABLE II—concl'd.

Composition of the diet of various families. (Oz. per consumption unit per day).

GROUP IV.							
Provision merchant.							
Raw, milled rice	..	11.31	Brinjal	..	0.45	Jack-nuts (<i>Artocarpus integrifolia</i>)	0.17
Parboiled rice	..	2.56	Pumpkin	..	0.30	Coco-nut ..	0.57
Dhal arhar ..	..	0.83	Lady's fingers (<i>Vibiscus esculentus</i>)	..	0.22	Gingelly oil	0.85
Black gram ..	..	0.52	Snake gourd (<i>Trichosanthus anguina</i>)	..	0.21	Gingelly seeds	0.10
Bengal gram ..	..	0.28	Potato	..	0.21	Whole milk	9.50
Mung dhal (<i>Phaseolus radiatus</i>)	..	0.05	Drumstick ..	..	0.13	Curds ..	0.09
Tamarind ..	..	0.50	Drumstick leaves	..	0.09	Chee ..	0.90
Coriander seeds	..	0.15	Plantain (fruit)	..	0.27	Jaggery ..	0.35
Onions ..	..	0.06	Plantain (stem)	..	0.12	White sugar	0.90
			Mangoes ..	..	0.16		

ANALYSIS OF DIETS.

Table I shows intake of calories, proximate principles and minerals, and the percentage of total calories, derived from cereals. Families are arranged in descending order of calorie intake.

In Table II, the composition of the diet of a number of families, as regards actual foodstuffs, is given. The families are chosen more or less at random out of the 4 groups as follows: Group I, families 1 and 7. Group II, family 3. Group III, families 3, 7, 14, 22, and 29. Group IV, provision merchant.

The average number of foods entering into the diets of the various groups during the period of investigation was as follows:—

GROUP I: 12 (range 8 to 18).

GROUP II: 20 („ 18 „ 24).

GROUP III: 11 („ 4 „ 24).

In Group IV the number of foods in the 3 diets was 33, 26 and 28 respectively. It is to be observed that many of the foods listed in the various diets shown in Table II were consumed only once or twice during the 20 days of the investigation.

Except in the case of a few prosperous families, foods other than cereals were consumed in very small quantities, as is apparent from Table II. In Group I the diet was composed almost exclusively of home-pounded parboiled rice; four of the group consumed a little ragi (millet). In Group II also, home-pounded parboiled rice formed a large proportion of the diet, but other foods were more abundantly present than in the diet of Group I. Group III ate both roughly milled parboiled rice and millet (usually cholam, less commonly cambu), the cereals in most instances providing 80 to 90 per cent of total calories.

Milk.—Milk products were absent from the diet of 31 out of the 44 families during the period of investigation. The remainder, including all the more prosperous families, consumed milk products in the form of milk, butter-milk, curds, or ghee. Quantities were as follows:—

GROUP II.

Family.	Product.	Quantity per consump- tion unit per day (oz.).
1	Whole milk ..	3.10
2	Curds ..	0.13
3	Whole milk ..	0.13
4	Whole milk ..	0.42
	Curds ..	1.10
	Butter-milk ..	0.48

GROUP III.

Family.	Product.	Quantity per consump- tion unit per day (oz.).
8	Butter-milk ..	5.25
9	Butter-milk ..	0.16
11	{ Whole milk ..	3.72
	{ Curds ..	2.38
13	{ Whole milk ..	14.00
	{ Curds ..	3.50
	{ Ghee ..	0.68
17	Whole milk ..	0.34
26	Whole milk ..	2.17

GROUP IV.

1	{ Whole milk ..	1.80
	{ Curds ..	3.10
2	{ Whole milk ..	1.35
	{ Curds ..	5.40
	{ Ghee ..	0.50
3	{ Whole milk ..	9.50
	{ Curds ..	0.09
	{ Ghee ..	0.90

It will be observed that only 3 families out of the whole group consumed ghee. The term 'curds' is applied to curdled milk, and the nutritive value of curds may be taken as equal to that of a similar quantity of whole milk.

Animal foods other than milk.—Only 3 out of the 44 families abstain from flesh foods because of religious conviction. The remainder eat mutton, fowl, fish, eggs, etc., when they can get these.

The majority of the families in Group I, during the short period of investigation, were eating small crabs caught in the local 'tanks' (irrigation ponds which fill up after the rains and dry up slowly during the non-rainy season). The supply of crabs lasts only a short time and this type of food is an unimportant part of the diet. One family during the period of investigation had a single meal which included a little chicken, and another including mutton. The families in this group consumed

also very small quantities of fish, averaging 0.25 oz. per day. Three of the families in Group II consumed fish in roughly similar quantities.

In Group III the diet of 10 families contained almost negligible amounts of fish, mutton, or chicken, the highest intake of foods of this nature being 0.80 oz. per c. u. daily. One family consumed a few eggs. No animal food apart from milk was included in the diet of Group IV.

Taking into consideration the small intake of milk and the virtual absence of meat and fish from the diet, it is obvious that the figures of protein intake given in Table II represent, for practical purposes, protein of vegetable origin.

Pulses.—The absence of animal protein from the diet, and the large proportional intake of cereals, make pulses an important ingredient in the diet of South Indian villagers. All the families under investigation consumed pulses, though some families, notably those in Group I, consumed only very small quantities. The most common pulse was dhal arhar (*Cajanus indicus*). The following indicates average consumption of pulses in the various groups :—

Mean

GROUP I : 0.26 oz. (range 0.08 to 0.36).

GROUP II : 1.29 „ („ 0.91 „ 1.98).

GROUP III : 1.32 „ („ 0.12 „ 3.97).

GROUP IV : 2.33 „ („ 1.30 „ 3.32).

Vegetables.—The Chinese are said to consume green leafy vegetables in large quantities, which results in the partial 'balancing' of their otherwise deficient diet. The same cannot be said of the South Indian families studied in the present investigation, at any rate during the period of inquiry. Only 4 of the families in Group I consumed green leafy vegetables, the highest consumption being 0.27 oz. per c. u. per day. In Group II consumption in 3 families averaged 0.30 oz., the other family consuming none. In Group III, 16 out of 31 families made use of leafy vegetables, average consumption among these being 0.40 oz. Consumption was low in Group IV (average of 3 families : 0.23 oz.).

Of non-leafy vegetables, brinjal (egg-plant) was the most commonly eaten. In Groups II, III, and IV it was absent from the diet of only 4 families ; Group I did not consume it at the season of inquiry. Other non-leafy vegetables eaten included drumstick, ridge gourd (*Luffa acutangula*), snake gourd, radish, onions, colacasia, and lady's fingers.

Omitting 4 families in Group III which did not use vegetables of this nature, average consumption in the various groups was as follows :—

GROUP I : 0.40 oz.

GROUP II : 2.70 „

GROUP III : 1.30 „

GROUP IV : 2.30 „

With regard to Group I, the missionary who kindly helped with the inquiry wrote a few weeks later as follows : ' You will notice the shortness of vegetables in the list. Since the recent rains the people have added some more vegetables to their diet '.

Fruit.—Fresh fruit was included in the diet of 17 families, the fruit most commonly consumed being plantain, green or ripe. The average intake of fruit in these families was 0.48 oz. per c. u. per day. The more prosperous families in Group IV consumed, in addition to plantains, mangoes in small quantities.

Tamarind (the dried fruit of the tree which commonly grows by the roadside in India) was present in the diet of all families, average intake being 0.55 oz. The consumption of the majority of families lay close to this figure. It would appear that a certain quantity of tamarind is included in the diet of even the poorest Indian peasant.

Vegetable oils.—Twenty-eight families consumed vegetable oil (gingelly), the average intake being 0.62 oz. Coco-nut, present in the diet of a few families, is included in this figure, having a high fat content. The diet of the remainder contained no vegetable oil or coco-nut.

EXAMINATION OF SCHOOL CHILDREN.

In order roughly to assess the state of nutrition associated with the dietary conditions observed in the Mayanur district, 323 children (boys), of ages ranging from 4 to 13, were examined in local schools. Some of these were drawn from the families of Group III; the remainder belonged to families consuming a similar kind of diet. It seems reasonable, in attempting to relate 'state of nutrition', and the results of village diet surveys in sample families, to examine a group of children in the area covered, irrespective of whether they all belong to the families included in the survey.

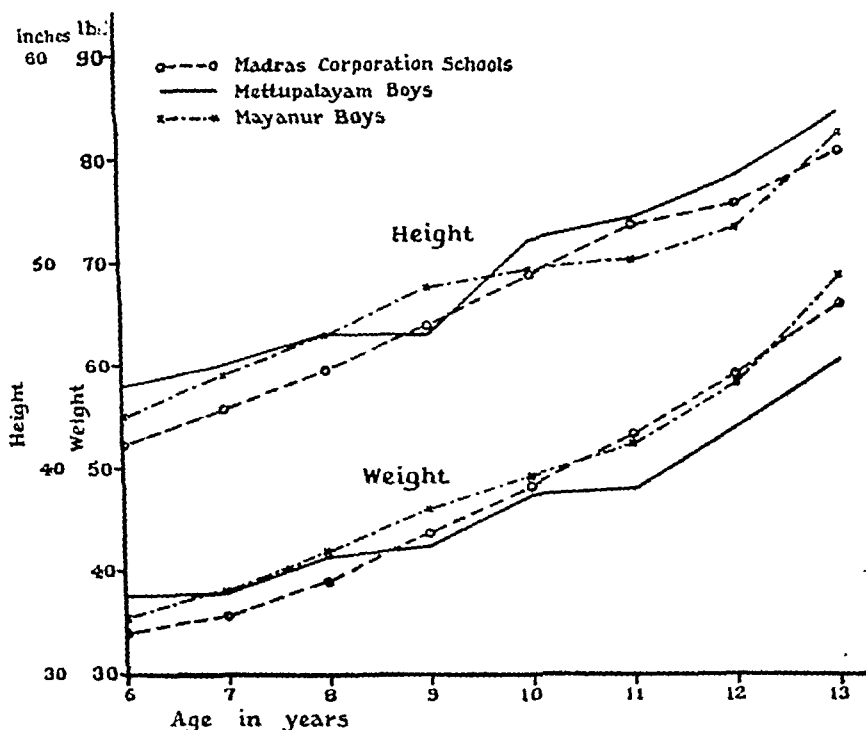
Heights and weights were taken, and the incidence of certain symptoms of food deficiency disease—phrynoderma, angular stomatitis, and Bitot's spots (xerophthalmia)—was observed. The method of clinical examination was that followed by Aykroyd and Rajagopal (1936) in carrying out surveys in South Indian towns.

Height and weight averages in the age groups 6 to 13 were as follows :—

Age.	Number of boys.	Height (inches).	Weight (lb.).
6	33	42.5	35.5
7	40	44.6	37.9
8	45	46.6	41.9
9	53	48.8	46.0
10	49	49.5	49.1
11	35	50.0	52.4
12	33	51.5	59.3
13	22	55.9	68.5

In Chart 1, these height and weight averages are compared with those of two other groups (boys) : Madras Corporation schools (1934-1935), a very poor urban group in which a high incidence of malnutrition is reported ; a semi-urban group in a small South Indian town, Mettupalayam (1936). It will be observed that the Mayanur averages correspond very closely with the Madras averages. In the Mettupalayam group, height averages are somewhat larger in the older age groups, and weight averages somewhat smaller. In a previous paper (Aykroyd and

CHART 1.



Rajagopal, *loc. cit.*) height and weight averages of South Indian boys were compared with those of boys in other countries, and it was suggested that the low 'weight for height' of Indian children, which is shown by the Mayanur group, is partly due to malnutrition.

In Table III, the percentage incidence of phrynoderma, Bitot's spots, and angular stomatitis in the Mayanur district children is given, and compared with that found in 3 South Indian towns (Aykroyd and Rajagopal, *loc. cit.*). It will be

noted that the incidence of these conditions was less than that observed in the previous investigation.

TABLE III.

Incidence of phrynoderma, angular stomatitis, and Bitot's spots.

Area.	Number examined.	Number showing phrynoderma.	Percentage.	Number showing angular stomatitis.	Percentage.	Number showing Bitot's spots.	Percentage.
Mayanur district	323	1	0.3	23	6.6	14	3.8
Coonoor ..	777	67	8.6	68	8.7	16	2.1
Mettupalayam ..	377	29	7.7	48	12.7	21	5.6
Calicut ..	426	2	0.5	43	10.1	35	8.2

DISCUSSION.

How far are the diets of the various families sufficient in quantity? It is of little use to compare observed calorie intake with 'generally accepted standards', since such standards are European or American in origin. Only 4 families out of 44 consumed the 3,000 calories per consumption unit which are traditionally the requirement of 'an average man at average work'.

The families in Group I had not enough to eat. We know this, because they told us so. A complaint of hunger is perhaps better evidence of insufficient calorie intake than a textbook deduction. Average calorie intake in this group per consumption unit per family was 1,664; range 2,026 to 1,184. We may, therefore, take it that the daily calorie requirements of groups of South Indian village families, assessed in terms of the conventional scale used here, exceed the mean intake of this group.

Two facts regarding these families may be mentioned in passing. We were informed that they displayed 'an obvious lack of energy and initiative' which scarcely seems remarkable in the circumstances. Some time after the inquiry the missionary who kindly co-operated wrote: 'I have been finding a condition of cedema in very young children and have come to the conclusion that it is due to excess of crude salt. The salt is not cooked in the rice but sprinkled over it when the rice is being eaten. These children have improved at once when salt-free diet has been given'. It is possible that these children had a tendency towards cedema related to the 'hunger' or 'famine' cedema so commonly observed in Central and Eastern Europe during and just after the war."

The mean calorie intake of Group III is obviously no guide to the requirements of South Indian population groups, since the series included families with 'hungry mouths'. Calorie requirements can be deduced from intake only when intake is unrestricted by poverty or other circumstances. It is worth noting, however, that mean calorie intake in this group was greater than the mean intake (1,940) of 16 peasant families studied by Nicholls (*loc. cit.*) in Ceylon and that of 15 Javanese families, which was 2,116 (Ochese *et al.*, *loc. cit.*). Nicholls suggests that 'a diet of the value of about 2,200 calories is sufficient for the requirements of an agricultural labourer belonging to the smaller races of the Tropics, provided the diet is well balanced in necessary constituents'.

Five families in our investigation stood out from the rest in being in the possession of an income obviously sufficient to allow appetite to dictate the amount of food intake. The calorie intake of these was as follows:—

GROUP II, family	1	: 2,473
GROUP III „	13	: 2,453
GROUP IV „	1	: 2,394
GROUP IV „	2	: 2,963
GROUP IV „	3	: 2,464

Mean : 2,549

The mean figure is very much below that given by Wilson *et al.* (*loc. cit.*) as average intake per 'mean value' in 10 well-to-do Bengali Hindu families (3,411). These workers used Lusk's scale.

We have attempted, in a rough and ready fashion, to work out the minimum energy expenditure budget of a South Indian peasant. According to Rahman (1936) the average basal heat production per hour of a group of male students in Hyderabad was about 60 calories, some 7 to 8 per cent below American standards. Mason and Benedict (1931) recorded an average of 44 calories in 54 Indian women (students and teachers) in Madras—an average deviation from the Aub and du Bois standards of 17.2 per cent.

During sleep the metabolism rate has been reckoned as 10 per cent below the basal. Experiments on European and American subjects have shown that any form of manual labour raises metabolism at least 3 times above the basal, while 'sitting at rest' raises it about 1.3 times. Assuming that the South Indian peasant spends 8 hours of the 24 in sleep, 8 hours at work, and 8 hours 'sitting at rest', his energy expenditure budget would work out as follows:—

8 hours sleep at ..	..	54 calories per hour :	432 calories.
8 „ work at ..	..	180 „ „ :	1,440 „
8 „ 'sitting at rest' ..	..	86 „ „ :	688 „
Total :			2,560 „

This seems a minimum budget, since expenditure at work is reckoned at the figure corresponding to expenditure in light manual occupations (e.g., carpentry). No figures are available for the energy expenditure involved by agricultural labour,

but in general it has been found that agricultural labourers in Europe and America have a high calorie intake. For example, the average calorie intake per consumption unit of a series of Roumanian peasant families was found to exceed 3,000 (Aykroyd, Alexa and Nitzulescu, 1935). The item of 688 calories for 8 hours 'sitting at rest' seems also a minimum, since peasants do not occupy all the waking hours not spent at work in this fashion.

Reckoned on similar basis, the daily energy expenditure of a woman with a basal metabolism rate per hour of 45 calories would work out at 1,900 calories. No data exist about the basal metabolism of Indian children.

All things considered, we are inclined to estimate the *minimum* daily calorie requirements per consumption unit of South Indian peasant families as lying in the neighbourhood of 2,500. If the diet of a labourer falls much below this figure, adjustment occurs as follows: basal metabolism is reduced, the body functioning, as it were, at a lower level of vitality, and energy output is necessarily made consonant with energy intake, since energy cannot be created. In simple terms, the under-fed labourer is lethargic and his output of work is small. A level of food intake which permits only a languid existence cannot be described as normal.

We are making use of the figure 2,500 in practical nutrition work, e.g., in drawing up diet schedules for institutions. It represents, of course, an average, and all individual cases falling somewhat below it need not be regarded as under-fed. If this figure is accepted, it appears that calorie intake in Groups I and II (3 families in the latter group) is definitely insufficient. In Group III, the mean approaches the standard minimum requirement, but conceals (since the intake of a number of families was in excess of the minimum) under-nutrition in a considerable proportion of families. The statement seems justified that one-third to one-half of the group of 44 families studied did not consume enough food during the period of investigation.

Judged by conventional standards, protein and fat intake was low. There was an almost complete absence of protein and fat of animal origin. The level of protein and fat intake in the poorer families was largely dependent on the nature of the cereal consumed, the protein and fat content of millet being higher than that of rice. The very low level of fat intake in Group I is due to the fact that the diet was mainly composed of rice and insufficient in quantity. Little can be said regarding the adequacy of calcium intake, except that it falls below conventional requirements in Groups II, III, and IV. The inclusion of millet, particularly ragi, in a largely cereal diet greatly raises calcium content, but Ranganathan (1935) has observed that the calcium in ragi and cambu is not well assimilated by rats. Phosphorus intake appears adequate; it is, however, to be observed that a considerable loss of phosphorus occurs on cooking from diets largely based on rice. Intake of iron appears high in relation to the general qualitative insufficiency of the diet; this may be ascribed to the fact that the iron content of many foods analysed in the laboratories has been found to be high, possibly because they originated in a district where the soil is rich in iron. The problem of iron requirements has been complicated by the observation that the iron contained in many foods is partially 'unavailable'.

Vitamin A was lacking, or present in infinitesimal quantities, in the diet of 39 of the 44 families. The highest intake, 500 γ ($\gamma=0.001$ mg.) per c.u. per day, was

that of family I, Group IV, which consumed milk products in reasonable quantities. Carotene intake in Groups I, II, and III, and vitamin-A and carotene intake in the families comprising Group IV, were estimated as follows:—

GROUP.	Mean carotene intake (γ per c. u. per day).	Range (γ).
I ..	669	102 to 1,052
II ..	632	283 to 1,363
III ..	828	182 to 3,544

GROUP.	Carotene intake (γ per c. u. per day).	Vitamin-A intake (γ per c. u. per day).
IV { Family 1	440	500
" 2	1,098	200
" 3	1,400	256

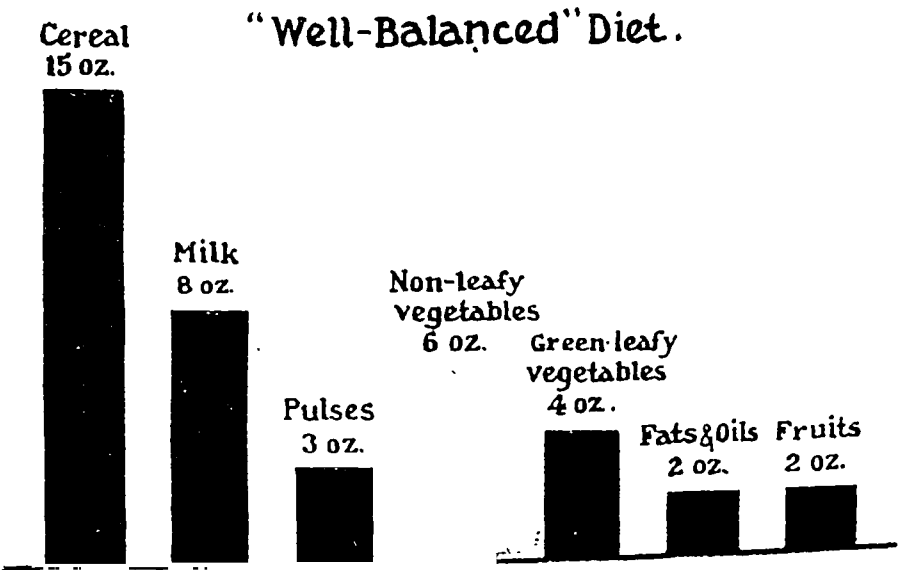
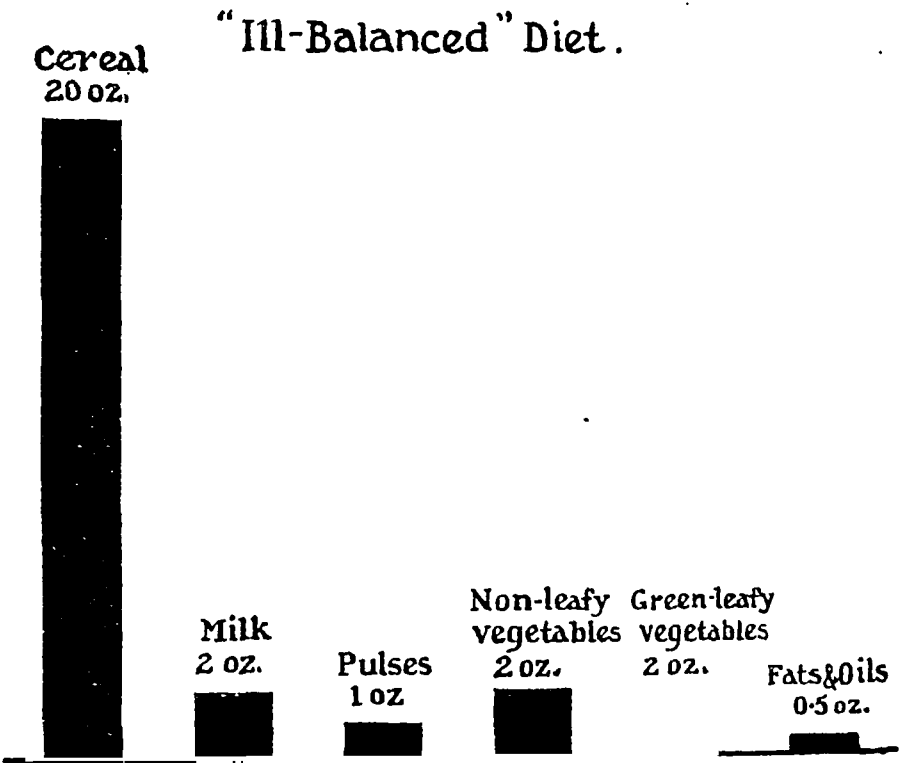
Estimates of vitamin-A and carotene intake are based on spectrographic assays carried out by Mr. N. K. De in these Laboratories. They are rough estimates, particularly as regards carotene, because the carotene content of foodstuffs is influenced by many factors (De, 1936), and the foodstuffs richest in carotene—green leafy vegetables—are those the intake of which it is most difficult to record accurately in diet surveys. In a previous paper (Aykroyd and Krishnan, 1936) we described the occurrence of xerophthalmia in a high percentage of children admitted to labour camps from a famine area, whose diet contained about 700 γ of carotene daily. The view was expressed that this quantity is insufficient to cover the requirements of children. Bitot's spots were observed in 14 (3·8 per cent) of the group of children examined in the present investigation. We have found, in extensive investigations in children's hostels, that the percentage incidence of Bitot's spots and the apparent vitamin-A activity of the diet do not always run parallel, but in general this eye lesion appears to be associated with the consumption of a diet deficient in carotene and vitamin A. Both from the estimated quantities of these food factors consumed and the presence of xerophthalmia in the children examined, it may be concluded that the diet of the majority of families was somewhat deficient in vitamin-A activity.

Similarly, a deficiency of vitamin B₂ is shown by the presence of 'angular stomatitis'.

Beri-beri does not appear to be prevalent in the areas in which the surveys took place, though endemic in other parts of the Madras Presidency where raw milled rice is the chief staple. It has long been known that beri-beri is rarely or never seen in populations consuming home-pounded or milled parboiled rice, or in millet eaters. If very poorly fed village folk of the type investigated were to discard their parboiled rice or millet in favour of raw milled rice, beri-beri would doubtless

soon appear. Although no signs of scurvy were observed, many of the diets seem to be deficient in vitamin C.

CHART 2.



It is difficult to say how far the families studied were typical of South Indian peasants in general. To our collaborators in Chingleput and Mayanur they appeared typical of millions of village families throughout the country. It is clear that if Group I, which may without exaggeration be described as half-starved, is representative of a large group, the problem of under and malnutrition in South India is more serious than has yet been realized. Variations in diet at different seasons of the year require further study. We propose to carry out investigations at other times of the year on the groups studied here or on other similar groups.

There is reason to suppose that in India malnutrition is more marked among the poor of the towns and cities than among the poor of the countryside. The incidence of symptoms of food deficiency disease in the Mayanur district boys was lower than that in Coonoor, Mettupalayam and Calicut boys, and apparently also lower than that reported in Madras Corporation schools. It is our experience that 'state of nutrition' is better when the diet is based on millet, or at least contains a fair proportion of millet, than when the only cereal eaten is milled rice, as is often the case in towns and cities. Village populations have the further advantage over town populations that, when they do consume rice, they usually consume it in roughly milled form, often parboiled. The families in Group I, though they consumed home-pounded rice, were in a poor state of nutrition; their condition would be much worse if their staple cereal was highly milled. While living standards in India remain at the present level, we must view with alarm any extension of mechanical rice milling in rural areas.

Though in general the poor in the villages may suffer less severely from malnutrition than the urban poor, the present investigation emphasizes the deficiencies of the South Indian diet, notably the lack of milk and other 'protective' foods. Some months ago, a chart was prepared in the laboratory for propaganda purposes, illustrating, in terms of South Indian food habits, the difference between a 'well-balanced' and an 'ill-balanced' diet. The chart is reproduced (Chart 2) opposite.

Both diets as described would yield 2,600 calories, roughly adult daily requirements: the cost of the well-balanced diet, at current retail prices, is about Rs. 5 per adult per month; that of the ill-balanced diet, about Rs. 3-8. The diet of the families studied corresponds roughly in quality to the 'ill-balanced' diet, except that observed intake of milk and vegetables was even smaller than that shown diagrammatically. The chart may, however, be used to show the changes in agricultural production required to 'balance' the South Indian diet. Whether, given the existing standard of living and the fact of increasing population, changes in the direction of the well-balanced diet (e.g., a larger milk supply) are possible, is a fundamental problem which nutrition and agricultural research workers in India must face.

SUMMARY.

1. An investigation of the diet of various groups of villages in South India was carried out, the period of survey being 20 days. An account is given of the methods by which data were collected.

2. Intake of calories, proximate principles and minerals per consumption unit per day in the various families is tabulated and the composition of a number of characteristic diets as regards actual foodstuffs is described.

3. An account is given of the consumption of milk, meat, vegetables, fruit, and vegetable oils.

4. Examinations of a number of school children drawn from families whose diet was investigated, and similar families in the same district, were carried out. The height and weight averages of the children were compared with those of other malnourished groups in South India. The incidence of symptoms of deficiency disease was rather lower than that observed in various urban areas.

5. The adequacy of the calorie intake in the various groups is discussed and the tentative conclusion is reached that one-third to one-half of the families were under-fed. The various qualitative deficiencies in the diet are pointed out.

6. There is reason to suppose that less malnutrition is found in millet-eating areas than in rice-eating areas. The diet of rural populations is often better than that of town populations because the former consume unmilled rice or millet and the latter usually highly milled rice.

In conclusion, we must thank workers attached to the Indian Red Cross Society, Madras, the Church of Scotland Mission, Chingleput, and the Servants of India Society Rural Centre, Mayanur, for their painstaking and enthusiastic co-operation in these investigations.

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SURVEY OF THE NUTRITIVE VALUE OF INDIAN FOODSTUFFS.

Part I.

THE CHEMICAL COMPOSITION OF 200 COMMON FOODS.

BY

S. RANGANATHAN,

A. R. SUNDARARAJAN,

AND

M. SWAMINATHAN.

(*Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.*)

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DURING recent years increasing attention has been given to the problem of nutrition in India, and its importance is now generally realized. Knowledge of food values is the essential basis of almost all public health nutrition work; there is a considerable demand for such knowledge, not only on the part of medical and public health workers, but also on the part of the educated public. The rational planning of diets in institutions, for example, is impossible without the help of an authoritative table of food values. Further, such data are of importance in connection with the formulation of agricultural policies designed to improve the diet of the people. Finally, detailed knowledge of food values is required for developing the research programme of the laboratories, which includes diet surveys throughout the country.

Up to the present no comprehensive survey of Indian food values has been carried out. McCay (1910), Sahasrabuddhe (1925), and Stewart *et al.* (1931) have published tables based on the chemical analysis of some common Indian foods; their work does not, however, cover a wide range. McCarrison's (1929) primer, 'Food', which has been widely used in planning diet schedules, provides data about only a limited number of foods, and includes no information about mineral content. In certain other Eastern countries complete and authoritative tables are already in existence; mention may be made of Rosedale's (1935) 'Chemical Analysis of Malayan Foods' and Hermans's (1932) 'Food Values', published by the Philippine Bureau of Science. Considerable attention is being given to the same subject in China and Ceylon.

The present investigation forms part of a systematic foodstuffs survey now proceeding at the Coonoor Laboratories. It deals with the results of the chemical analysis of some 200 foodstuffs, covering most of the cereals and pulses, vegetables (leafy, roots, and tubers, and others), nuts and oil seeds, fruits, condiments and spices, and a few flesh foods in common use.

COLLECTION OF MATERIAL.

The foodstuffs analysed were mostly obtained in the local market. Foods which may be described as common Indian foods, consumed throughout the country, originated in the majority of cases in the neighbouring plains of the Coimbatore district; others of a kind less widely used in India (e.g., European vegetables, such as lettuce) were largely grown in the neighbourhood of Coonoor, 6,000 feet above sea-level. Among the foods analysed were some from other parts of India, including North India. The place of origin of each foodstuff is indicated in column 3 of the tables. The edible portion of the foodstuff, in as fresh a state as possible, was used for the analysis; the results of analysis relate only to the edible portion.

METHODS OF ANALYSIS.

The chemical analysis of the foodstuffs includes a determination of the following: moisture, the three 'proximate principles' (protein, fat, and carbohydrate), total mineral matter, crude fibre, and the three minerals usually considered of importance in practical dietetics: calcium, phosphorus, and iron. The methods employed were mostly standard ones and, briefly described, were as follows:—

1. *Sampling*.—About 100 grammes to 150 grammes of the foodstuff, freed as far as possible from extraneous matter, are ground to a fine powder and passed through a fine-mesh sieve. Where such powdering cannot be done, as in the case of fruits, vegetables, etc., a representative sample is obtained, and fractions used for the determination of the following constituents.

2. *Moisture*.—About 2 grammes to 3 grammes of the test sample are dried to constant weight in an electric hot-air oven maintained at 100°C., and the loss in weight consequent on drying is reckoned as moisture.

3. *Total mineral matter*.—About 25 grammes to 100 grammes of the test material, the amount depending on the nature of the foodstuff, are heated slowly in a platinum dish over a Bunsen flame till complete carbonization takes place, then removed to an electric muffle furnace and ashed to constant weight. The ashing was carried to the stage at which there were no enclosed carbon particles. The weight of ash is reckoned as total mineral matter.

4. *Ether extractives*.—Five grammes to 20 grammes of the test material are weighed into a fat-free thimble slowly dried in a hot-air oven till fairly dry. It is then extracted with dry ether for about 20 to 24 hours in a Soxhlet's apparatus with ground-glass joints. The extract is freed from the solvent, dried to constant weight, and reckoned as ether extractives.

5. *Crude fibre*.—The residue left over from (4) is utilized for the determination of crude fibre, the method being that adopted by the Association of Official Agricultural Chemists (1930).

6. *Proteins*.—Total nitrogen is determined by the usual Kjeldahl's method and the protein content calculated therefrom by multiplying the nitrogen figure by 6.25.

7. *Carbohydrates*.—The carbohydrate content is obtained by subtracting the sum of moisture, total mineral matter, ether extractives, crude fibre, and proteins, from 100.

8. *Calorific value*.—This is obtained by multiplying the sum of carbohydrates and proteins by 4 and adding to it the figure obtained by multiplying the ether extractives by 9,

i.e., calorific value = 4 (carbohydrates + proteins) + 9 × ether extractives.

9. *Fractional analysis of the ash*.—The ash obtained under (3) is gently moistened with a few drops of distilled water and then 5 c.c. to 10 c.c. of pure, iron-free hydrochloric acid are added. The solution is boiled for a few minutes and filtered into a 100 c.c. measuring flask. The filtrate and the washings of the filter-paper, collected in the same flask, are made up to mark; aliquots are taken for the determination of calcium, phosphorus, and iron.

10. *Calcium*.—Ten c.c. to 25 c.c. of the solution prepared as under (9) are rendered strongly alkaline with ammonia. Ammonium oxalate is then added in excess and the solution brought to the boil. While still hot, the solution is rendered strongly acid with glacial acetic acid. The precipitate of calcium oxalate is allowed to settle over-night. It is then washed till free from ammonium oxalate with boiling distilled water, dissolved in hot, dilute sulphuric acid, and titrated against deci- or centi-normal potassium permanganate. Whatman No. 44 filter-paper is used.

11. *Phosphorus*.—The volumetric method (Official) recommended by the Association of Official Agricultural Chemists (*loc. cit.*) is employed. Ten c.c. to 25 c.c. of the solution described under (9) are used for this estimation. Three per cent potassium nitrate solution is used for washing the precipitate of phosphomolybdate, instead of distilled water.

12. *Iron*.—Iron is estimated colorimetrically by the potassium thiocyanate method, the colour obtained being taken up by extra pure amyl alcohol (Farrar, Jr., 1935). The solution prepared as under (9) is employed except in the case of vegetables, fruits, etc., with acid juices, which have, in the preparation of the original sample, been cut with a steel knife. In such cases about 5 grammes of the material, obtained without making use of any metallic blades, are ashed, and the ash brought to solution with iron-free hydrochloric acid; an aliquot of this solution is taken for iron estimation. To this aliquot, taken in a separating funnel, a drop or two of concentrated, iron-free nitric acid is added to oxidize any ferrous salt. Five c.c. of amyl alcohol are then added, followed by 5 c.c. of 20 per cent potassium thiocyanate solution. The liquid is shaken well and the amyl alcohol layer separated off; this extraction of the thiocyanate colour is done more than once, usually thrice; the amyl alcohol layers in the different extractions are mixed, made up to volume in either a 10 c.c. or a 25 c.c. measuring flask, depending on the intensity of the colour, and matched in a colorimeter against suitable standards treated likewise. Blank estimations are carried out during each set of iron determinations to ensure the freedom of the reagents employed from iron, as even reagents labelled 'extra pure' not seldom contain traces of iron.

TABLE Ia.

Cereals.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Arrow-root flour (West Indian).	<i>Maranta arundinacea</i>	Local bazaar	16.51	0.17	0.06	0.12	..	83.14	0.009	0.017	1.00	333.8	
'Bajra' or 'cambu'	<i>Pennisetum typhloideum</i>	Coimbatore	12.41	11.59	4.90	2.95	1.22	67.14	0.049	0.352	8.84	359.9	
Barley ..	<i>Hordeum vulgare</i>	Local	12.48	11.47	1.28	1.51	3.92	69.34	0.025	0.225	3.70	334.8	Without outer husk.
'Cholam'	<i>Sorghum cernuum</i>	Bellary	12.31	10.10	1.89	1.18	..	74.52	0.014	0.246	7.44	355.5	
" yellow	<i>S. durra</i>	Coimbatore	11.65	8.84	1.47	1.56	..	76.48	0.040	0.309	6.10	344.5	
" 'irungu'	"	"	12.12	10.51	2.96	1.56	..	..	0.017	0.300	5.72	..	
" 'irungu'	<i>S. vulgare</i>	"	11.42	12.23	1.50	2.73	..	72.12	0.038	0.274	5.47	350.9	
Italian millet	<i>Setaria Italica</i>	Local bazaar	12.31	12.31	4.65	3.24	7.95	60.62	0.026	0.287	6.25	333.6	
'Koota'	<i>Fagopyrum esculentum</i>	Bijoor (U. P.)	11.33	10.26	2.48	2.42	8.60	65.03	0.068	0.299	13.20	322.4	
Maize, Indian corn (tender green).	<i>Zea mays</i>	Local	79.36	4.33	0.46	0.65	..	15.20	0.005	0.102	0.68	82.3	
'Mukhana'	—	Bijoor (U. P.)	12.75	9.70	0.14	0.52	..	76.89	0.016	0.091	1.38	347.6	
Oatmeal	<i>Avena sativa</i>	Local bazaar	10.68	13.55	7.59	1.78	3.46	62.94	0.045	0.384	3.81	374.3	
'Pani varagu'	<i>Panicum miliaceum</i>	Coimbatore	11.85	12.49	1.06	3.39	2.16	69.05	0.014	0.333	5.72	335.7	
'Ragi' ..	<i>Eleusine coracana</i>	Mangalore	13.34	6.94	1.11	2.19	..	76.42	0.358	0.238	5.40	343.4	
" brown	"	Coimbatore	12.58	7.92	1.54	2.21	..	75.75	0.386	0.311	6.22	348.5	
" 'marua'	"	Darbhanga	13.22	6.44	1.22	2.33	..	76.79	0.257	0.268	4.53	343.9	
Rice, 'guddu'	<i>Oryza sativa</i>	Mangalore	12.19	9.06	0.41	0.67	..	77.75	0.007	0.104	5.57	351.0	
" 'maskoti'	"	"	12.20	7.83	0.25	0.85	..	79.07	0.004	0.165	4.65	349.1	
" 'suggi'	"	"	12.19	8.49	0.35	0.70	..	78.27	0.005	0.134	2.75	350.2	
" ..	"	"	12.63	8.50	0.39	0.86	..	77.62	0.010	0.277	6.14	349.0	
" raw, polished	"	Local bazaar	12.96	6.85	0.55	0.50	..	79.14	0.007	0.108	1.02	348.9	
" parboiled	"	"	13.29	6.44	0.64	0.77	..	78.86	0.007	0.149	2.22	347.0	
" white 'puttu'	"	"	12.97	7.51	0.37	0.40	..	78.75	0.008	0.081	3.33	348.4	
" black 'puttu'	"	"	12.26	7.69	1.27	1.25	0.65	76.88	0.012	0.241	4.93	349.7	
" beaten	"	"	12.23	6.63	1.15	1.73	..	78.26	0.016	0.217	8.00	349.9	
" puffed	"	"	14.68	7.46	0.12	3.41	..	74.33	0.024	0.162	6.15	329.1	
Sago ..	<i>Metroylon sago</i>	Local	12.20	0.24	0.17	0.30	..	87.09	0.010	0.006	1.27	350.9	
'Sannu'	<i>Panicum miliare</i>	"	11.46	7.70	4.71	4.83	7.60	63.70	0.015	0.364	7.08	328.0	
'Sannu' or 'kodu'	<i>Panicum crusgalli</i> var. <i>frumentaceum</i> .	Coimbatore	11.87	6.22	2.23	4.44	9.83	65.41	0.019	0.282	2.86	306.6	
'Sannu' millet	<i>Trapa distans</i>	"	13.76	13.43	0.83	3.07	..	68.91	0.069	0.143	2.35	336.8	
'Singham', dry	<i>Trapa distans</i>	Bijoor (U. P.)	11.67	8.66	0.44	3.07	..	78.76	0.020	0.079	0.33	353.6	
Vernicelli	<i>Paspalum scrobiculatum</i>	Coimbatore	12.77	8.31	1.37	2.92	8.97	65.66	0.039	0.242	5.17	308.2	
'Varagu' or 'kodu' millet.	"	"	12.77	11.77	1.45	1.49	1.20	71.30	0.054	0.315	5.34	345.4	
Wheat ..	<i>Triticum vulgare</i>	Mangalore	12.77	11.77	1.45	1.49	1.20	71.30	0.054	0.315	5.34	345.4	

TABLE 1b.

Pulses.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
I	2	3	4	5	6	7	8	9	10	11	12	13	14
Bengal gram ..	<i>Cicer arctinum</i>	Local bazaar	9.83	17.08	5.26	2.68	3.92	61.23	0.185	0.236	9.83	360.6	With husk.
" ..	"	Mangalore	12.17	16.19	4.36	3.01	3.92	60.35	0.194	0.444	9.83	345.4	"
Bengal gram, roasted	"	Local bazaar	11.17	22.54	5.17	2.19	..	58.93	0.067	0.311	8.89	372.4	Without outer husk.
' Bhettas ' ..	<i>Glycine hispida</i>	Darbanga	8.80	41.29	16.95	4.49	4.25	24.22	0.213	0.603	9.88	414.6	
Black gram ..	<i>Phaseolus mungo</i>	Local bazaar	10.87	23.95	1.38	3.39	..	60.41	0.200	0.367	9.80	358.7	Without outer husk.
Cow gram ..	<i>Vigna catiung</i>	"	12.00	24.56	0.69	3.23	3.75	55.77	0.073	0.485	3.81	327.5	
" ..	"	Coimbatore	11.72	23.63	1.06	3.02	3.75	50.82	0.081	0.387	6.67	331.3	
Field bean, dry ..	<i>Dolichos lablab</i>	Mysore	9.60	24.94	0.78	3.21	1.38	60.09	0.063	0.446	1.98	347.1	
" ..	"	Local bazaar	12.21	20.43	0.84	2.90	1.38	62.24	0.113	0.334	5.33	338.2	
Green gram ..	<i>Phaseolus radiatus</i>	"	10.42	23.96	1.26	3.57	4.10	56.69	0.143	0.281	8.40	342.8	With husk.
Horse gram ..	<i>Dolichos biflorus</i>	Mangalore	11.81	22.01	0.54	3.13	5.30	57.21	0.275	0.385	7.59	321.7	
' Khesari ' ..	<i>Cicer arietinum (Lathyrus sativa P.)</i>	Darbanga	9.98	28.22	0.57	2.99	..	58.24	0.107	0.496	5.63	351.0	
' Masur dhal ' (lentil)	<i>Lens culcitra</i>	Local bazaar	12.44	25.10	0.74	2.13	..	59.56	0.130	0.250	1.98	345.4	
Peas, dried ..	<i>Pisum sativum</i>	"	16.03	19.68	1.14	2.12	4.50	50.53	0.068	0.298	4.44	315.1	
" roasted ..	"	"	9.90	22.94	1.39	2.34	..	63.43	0.033	0.357	5.00	358.0	
' Rajmah ' ..	—	Lahore	12.03	22.89	1.31	3.22	..	60.55	0.256	0.408	5.84	345.6	
' Ravan ' ..	<i>Vigna catiung</i>	"	12.67	23.40	1.28	2.92	..	59.73	0.075	0.430	4.31	344.0	
Red gram ..	<i>Cajanus indicus</i>	Local bazaar	15.23	22.27	1.71	3.56	..	57.23	0.136	0.264	8.80	341.9	Without outer husk.
" ..	"	Mangalore	12.76	21.12	1.84	2.80	..	61.48	0.056	0.276	8.80	347.0	
Soya bean ..	<i>Glycine hispida</i>	Ahmedabad	8.08	43.22	19.50	4.63	3.72	20.85	0.237	0.689	11.59	431.8	

TABLE Ic.

Vegetables : leafy.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Other extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.
1	2	3	4	5	6	7	8	9	10	11	12	13
Amaranth (tender) (spined)	<i>Amaranthus gangeticus</i>	Local	85.78	4.90	0.50	3.05	..	5.77	0.500	0.100	21.40	47.2
" (tender)	<i>A. spinosus</i>	Coimbatore	85.00	3.00	0.25	3.61	..	8.14	0.800	0.052	22.91	46.8
Bamboo (tender shoots).	<i>Bambusa arundinacea</i>	"	87.06	3.92	0.09	1.40	..	7.53	0.016	0.088	0.11	46.6
Brussels sprouts	<i>Brassica oleracea bullata gemmifera.</i>	Local	84.57	4.68	0.46	1.02	..	9.27	0.045	0.082	2.33	59.9
Cabbage	<i>Brassica oleracea capitata</i>	"	90.20	1.75	0.11	0.61	0.95	6.38	0.034	0.046	0.76	33.5
Celery	<i>Apium graveolens rapaceum.</i>	"	81.27	5.97	0.61	2.11	1.42	8.62	0.236	0.137	6.25	63.9
Coriander	<i>Coriandrum sativum</i>	"	87.90	3.20	0.64	1.65	..	6.52	0.137	0.058	9.97	45.0
Curry leaves	<i>Murraya koenigii</i>	Coimbatore	68.31	6.11	1.04	4.17	..	16.00	0.811	0.057	3.09	97.8
Drumstick	<i>Moringa oleifera</i>	"	74.98	6.65	1.73	2.27	0.87	13.50	0.437	0.071	7.00	96.2
Peas	<i>Trigonella scorpiogracum</i>	Local	81.78	4.86	0.86	1.57	1.04	9.89	0.472	0.047	16.90	66.7
Garden cress	<i>Lepidium sativum</i>	"	82.30	5.79	0.96	2.16	..	8.79	0.359	0.113	28.57	67.0
'Gogu'	<i>Hibiscus sabdariffa</i>	Godavari	86.22	1.70	1.07	1.02	..	9.99	0.181	0.038	5.38	56.4
Ipomoea	<i>Ipomoea reptans</i>	Local	90.33	2.90	0.40	2.11	..	4.26	0.113	0.048	3.90	32.7
Lettuce	<i>Lactuca sativa</i>	"	92.94	2.05	0.26	1.18	0.53	3.04	0.051	0.030	2.39	22.2
Mint	<i>Menha viridis</i>	"	82.99	4.81	0.59	1.56	1.95	8.10	0.204	0.077	15.56	57.0
'Neem' (mature)	<i>Azadirachta indica</i>	Coimbatore	59.44	7.09	1.04	3.36	6.19	22.88	0.514	0.080	17.14	129.2
" (tender)	"	"	59.35	11.56	2.97	2.61	2.22	21.28	0.127	0.189	25.30	158.1
Parsley	<i>Petroselinum sativum</i>	Local	68.42	5.93	0.97	3.17	1.80	19.71	0.392	0.195	17.86	111.3
'Agathi'	<i>Sesbania grandiflora</i>	Coimbatore	76.73	8.40	1.39	3.11	2.17	11.80	1.131	0.077	3.91	93.3
'Manthakali'	<i>Solanum nigrum</i>	Local	82.12	5.86	1.00	2.13	..	8.89	0.410	0.074	20.51	68.0
Spinach	<i>Spinacia oleracea</i>	Godavari	91.65	1.92	0.85	1.50	..	4.07	0.061	0.010	4.95	31.6

TABLE Id.

Vegetables: roots and tubers.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Beet root ..	<i>Beta vulgaris</i>	Local	83.81	1.65	0.05	0.75	..	13.74	0.195	0.057	0.98	62.0	Botanical name could not be traced.
Carrot ..	<i>Daucus carota</i>	"	86.00	0.92	0.07	1.08	1.18	10.75	0.082	0.030	1.52	47.3	
Colocasia ..	<i>Colocasia antiquorum</i>	"	73.05	2.95	0.10	1.72	..	22.18	0.038	0.143	2.13	101.4	
' Onthalai gasu ' * ..	—	"	84.40	1.24	0.05	0.34	..	13.97	0.013	0.024	0.53	61.3	
Paranip ..	<i>Pastinaca sativa</i>	"	72.41	1.33	0.27	1.12	1.68	23.19	0.048	0.041	0.40	100.5	
Potato ..	<i>Solanum tuberosum</i>	"	74.73	1.73	0.13	0.61	..	22.80	0.004	0.034	0.68	99.3	
Radish (pink) ..	<i>Raphanus sativus</i>	"	90.76	0.61	0.32	0.85	..	7.46	0.046	0.017	0.47	35.2	
" (white) ..	" "	"	94.41	0.70	0.06	0.68	..	4.25	0.054	0.025	0.40	20.3	
Sweet potato ..	<i>Ipomoea batatas</i>	Local bazaar	66.51	1.24	0.32	1.04	..	30.89	0.017	0.050	0.79	131.4	
' Tapioca ..	<i>Manihot utilisima</i>	"	59.37	0.68	0.20	0.98	..	38.77	0.045	0.039	0.92	159.6	
Yam, elephant ..	<i>Amorphophallus cam-panulatus</i> .	"	78.79	1.24	0.02	0.82	0.80	18.33	0.046	0.022	0.62	78.4	
" ordinary ..	<i>Typhonium trilobatum</i>	"	69.90	1.41	0.09	1.55	..	28.46	0.061	0.016	1.30	120.3	

* A root resembling tapioca.

TABLE Ie.
Other vegetables.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ruber extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorie value per 100 g.
1	2	3	4	5	6	7	8	9	10	11	12	13
Amaranth stem ..	<i>Anaranthus gangeticus</i>	Local	92.49	0.85	0.11	1.84	1.20	3.51	0.263	0.034	1.80	18.4
Artichoke ..	<i>Cynara scolymus</i>	"	77.27	3.64	0.15	1.75	1.20	15.99	0.120	0.101	2.29	79.9
Ash gourd ..	<i>Benincasa cerifera</i>	Coimbatore	96.01	0.41	0.06	0.34	..	3.18	0.025	0.017	0.45	14.9
Bitter gourd ..	<i>Momordica charantia</i>	"	92.39	1.64	0.15	0.76	0.80	4.26	0.023	0.065	2.22	25.0
" (small variety)	"	W. Godavari	83.16	2.87	0.98	1.43	1.70	9.86	0.045	0.144	9.35	59.7
Brinjal ..	<i>Solanum melongena</i>	Coimbatore	91.49	1.31	0.26	0.50	..	6.44	0.024	0.064	1.31	33.3
Broad beans ..	<i>Dolichos lablab</i> var. <i>lignosus</i> .	"	82.41	4.49	0.12	0.98	2.02	9.98	0.045	0.055	1.61	59.0
Calabash cucumber	<i>Lagenaria vulgaris</i>	"	96.34	0.16	0.14	0.49	..	2.87	0.015	0.003	0.69	13.4
Cauliflower ..	<i>Brassica oleracea botrytes</i>	Local	89.43	3.51	0.41	1.36	..	5.29	0.034	0.061	1.25	38.9
'Cho-cho' marrow	<i>Sechium edule</i>	"	92.53	0.70	0.11	0.41	..	6.25	0.138	0.026	0.62	28.8
Celery stalks ..	<i>Apium graveolens rapaceum</i> .	"	93.53	0.79	0.09	0.89	1.17	3.53	0.033	0.038	4.76	18.1
Cluster beans ..	<i>Cyamopsis psoralitoides</i>	Coimbatore	82.45	3.67	0.17	1.35	2.26	10.10	0.129	0.051	5.76	56.6
Colocasia stems ..	<i>Colocasia antiquorum</i>	"	93.44	0.34	0.34	1.20	0.59	4.09	0.062	0.024	0.50	20.8
Cucumber ..	<i>Cucumis sativus</i>	"	96.42	0.45	0.06	0.31	..	2.76	0.012	0.025	1.48	13.4
" ..	"	Godavari	90.86	0.72	0.13	0.93	..	7.36	0.037	0.062	2.12	33.5

TABLE 1e—concl.

Other vegetables—concl.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.
1	2	3	4	5	6	7	8	9	10	11	12	13
Snake gourd ..	<i>Trichosanthes anguina</i>	Coimbatore	94.06	0.46	0.31	0.70	..	4.47	0.045	0.024	1.28	22.5
Spinach stalks ..	<i>Spinacia oleracea</i>	Local	93.37	0.87	0.14	1.82	..	3.80	0.090	0.015	1.34	19.9
' Sundakai ' (dry) ..	<i>Solanum torvum</i>	Local bazaar	12.25	8.31	1.66	5.09	17.58	55.11	0.370	0.179	22.22	268.6
Tomato (green) ..	<i>Lycopersicum esculentum</i>	Local	92.81	1.88	0.06	0.68	..	4.57	0.016	0.039	2.37	26.3
Turnip ..	<i>Brassica rapa</i>	"	91.10	0.52	0.23	0.60	..	7.52	0.028	0.035	0.42	34.2
Vegetable marrow ..	<i>Cucurbita pepo</i>	Coimbatore	94.84	0.48	0.09	0.27	..	4.32	0.002	0.026	0.63	20.0

TABLE If.

Oil-seeds and nuts.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.
1	2	3	4	5	6	7	8	9	10	11	12	13
Almond ..	<i>Prunus amygdalis</i>	Local bazaar	5.23	20.75	58.92	2.90	1.70	10.50	0.225	0.492	3.47	655.3
Cashew ..	<i>Anacardium occidentale</i>	"	5.89	21.19	46.63	2.43	1.27	22.29	0.053	0.449	4.95	596.3
Coco-nut ..	<i>Cocos nucifera</i>	"	36.28	4.47	41.60	0.96	3.59	13.19	0.013	0.243	1.70	444.7
Gingelly ..	<i>Sesamum indicum</i>	"	5.08	18.33	43.26	5.20	2.88	25.25	1.453	0.574	10.54	563.7
Ground-nut ..	<i>Arachis hypogaea</i>	"	7.92	26.72	40.13	1.87	3.07	20.29	0.048	0.392	1.56	549.2
Ground-nut, roasted	"	"	4.04	31.54	30.76	2.28	3.07	19.31	0.045	0.435	0.29	561.2
Linseed ..	<i>Linum usitatissimum</i>	"	6.57	20.27	37.11	2.44	4.80	28.81	0.173	0.371	2.65	530.3
Mustard ..	<i>Brassica juncea</i>	"	8.46	22.04	39.64	4.19	1.80	23.87	0.488	0.704	17.88	540.4
Pistachios ..	<i>Pistacia vera</i>	"	5.58	19.81	53.51	2.75	2.10	16.25	0.136	0.431	13.70	625.8
Walnut ..	<i>Juglans regia</i>	"	4.53	15.64	64.49	1.84	2.60	10.90	0.095	0.384	4.76	686.6

TABLE Ig.

Fruits.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	(Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Apple ..	<i>Pyrus malus</i>	Local	85.94	0.31	0.08	0.27	..	13.40	0.013	0.021	1.68	55.0	Preserved.
Banana ..	<i>Musa sapientum</i>	Local bazaar	61.39	1.33	0.15	0.72	..	36.41	0.002	0.045	0.42	147.6	
Bullock's heart ..	<i>Anona reticulata</i>	Local	76.79	1.39	0.16	0.74	..	20.92	0.014	0.009	0.63	90.7	
Cape gooseberry ..	<i>Physalis peruviana</i>	"	82.69	1.78	0.17	0.59	3.19	11.58	0.006	0.058	1.80	55.0	
Dates (Persian) ..	<i>Phoenix dactylifera</i>	Local bazaar	26.11	3.04	0.16	1.31	2.07	67.31	0.067	0.076	10.58	282.8	
Figs ..	<i>Ficus carica</i>	"	80.80	1.31	0.23	0.64	..	17.02	0.055	0.026	1.16	75.4	
Grapes (blue variety)	<i>Vitis vinifera</i>	"	85.51	0.83	0.10	0.43	3.00	10.13	0.025	0.019	0.40	44.7	
Grape fruit (Triumph)	<i>Citrus grandis</i> var. <i>maxima</i> .	Local	92.04	0.71	0.03	0.20	..	7.02	0.017	0.021	0.18	31.2	
" (Marsh's seedless).	"	"	88.51	0.95	0.06	0.35	..	10.13	0.025	0.027	0.20	44.9	
Guava (country) ..	<i>Psidium guyana</i>	Coimbatore	76.07	1.46	0.19	0.81	6.90	14.57	0.013	0.040	1.04	65.8	
" (hill) ..	<i>P. catibianum</i>	Local	85.31	0.95	0.16	0.61	4.82	8.15	0.046	0.022	1.20	37.8	
Jack-fruit ..	<i>Artocarpus integrifolia</i>	Coimbatore	77.20	1.86	0.10	0.78	1.09	17.88	0.022	0.028	0.46	79.9	
Jambu fruit ..	<i>Eugenia jambos</i>	"	78.21	0.67	0.10	0.41	0.87	19.74	0.016	0.011	1.00	82.5	
* Korukapalli ..	<i>Pithecolobium dulce</i>	"	80.78	2.57	0.31	0.41	..	15.93	0.011	0.036	0.37	76.8	
Lemon ..	<i>Citrus medica</i> var. <i>limonum</i> .	Local	84.97	0.98	0.85	0.26	1.65	11.29	0.070	0.014	2.33	56.7	
Lime ..	<i>Citrus medica</i> var. <i>acidula</i>	"	84.57	1.49	0.97	0.64	1.25	11.08	0.091	0.021	0.29	59.0	
Mango (green) ..	<i>Mangifera indica</i>	Coimbatore	90.03	0.65	0.11	0.37	..	8.84	0.012	0.017	4.48	39.0	
" (type) ..	"	Salem	86.14	0.61	0.07	0.32	1.14	11.69	0.067	0.020	0.25	56.0	

" (Ankola)	"	Bombay	85-94	1-02	0-11	0-46	..	12-47	0-004	0-016	0-45	55-0
Melon, water	<i>Citrullus vulgaris</i>	Coimbatore	95-70	0-12	0-15	0-22	..	3-81	0-001	0-008	0-15	17-1
Orange ..	<i>Citrus aurantium</i>	Local	87-84	0-85	0-32	0-43	..	10-56	0-046	0-022	0-08	48-5
" ..	"	Punjab	91-39	0-85	0-08	0-51	..	7-17	0-023	0-025	0-24	32-4
" (Washington navel)	"	Local	89-76	0-66	0-10	0-31	..	9-17	0-020	0-020	0-18	40-2
" (Jaffa)	"	"	90-78	0-62	0-09	0-31	..	8-20	0-018	0-021	0-24	36-1
Palmyra fruit (tender)	<i>Borassus flabellifer</i>	Coimbatore	92-72	0-58	0-02	0-17	..	6-51	0-005	0-021	0-46	28-5
Papaya (ripe)	<i>Carica papaya</i>	Local	89-62	0-47	0-03	0-38	..	9-50	0-013	0-009	0-42	40-2
Peaches	<i>Amigdalus persica</i>	"	90-07	1-52	0-19	0-58	..	7-61	0-006	0-029	1-70	38-4
Pears (country)	<i>Pyrus communis</i>	"	86-92	0-18	0-05	0-27	1-00	11-58	0-006	0-011	0-68	47-5
Pears (English)	<i>P. Achras</i>	"	85-84	0-94	0-23	0-23	..	12-76	0-005	0-016	0-84	56-9
Pears, 'avocado' or butter fruit.	<i>Persca drymifolia</i>	"	73-55	1-68	22-31	1-05	..	0-91	0-011	0-076	0-71	215-7
Pineapple	<i>Ananas sativa</i>	Local bazaar	86-50	0-57	0-04	0-49	0-40	12-00	0-021	0-007	0-92	50-6
Plantain	<i>Musa paradisiaca</i>	"	73-35	1-06	0-08	0-72	..	24-79	0-011	0-031	0-50	104-1
" 'hill anai. kombu'.	"	Local	79-93	1-22	0-06	0-80	..	17-99	0-007	0-025	0-27	77-4
Plums ..	<i>Prunus domestica</i>	"	89-82	0-68	0-22	0-36	..	8-92	0-017	0-018	0-55	40-4
Pomegranate	<i>Punica granatum</i>	Local bazaar	78-00	1-63	0-04	0-67	5-10	14-56	0-009	0-066	0-27	65-1
'Ponche'	<i>Citrus decumana</i>	Local	88-10	0-63	0-04	0-51	0-60	10-12	0-028	0-027	0-04	43-4
Quince ..	<i>Cydonia vulgaris</i>	"	85-76	0-28	0-01	0-33	1-72	11-87	0-005	0-015	0-44	49-0
Raisins (preserved)	<i>Vitis vinifera</i>	Local bazaar	18-47	1-96	0-18	1-98	..	77-41	0-101	0-075	3-98	319-1
Strawberry (big)	<i>Fragaria grandiflora</i>	Local	87-82	0-71	0-16	0-38	1-14	9-77	0-026	0-026	1-75	43-5
'Tomato (ripe)	<i>Lycopersicum esculentum</i>	"	91-52	1-02	0-08	0-48	..	3-90	0-008	0-018	0-08	20-4
" (tree)	<i>Cyphonandra betacea</i>	"	82-72	1-46	0-21	1-12	4-15	10-34	0-013	0-029	0-71	43-3
Wood apple	<i>Fcavia elephanium</i>	Coimbatore	69-54	7-31	0-56	1-88	5-16	15-55	0-127	0-107	0-62	96-5
Tamarind (also referred to under condiments).	<i>Tamarindus indicus</i>	Local bazaar	20-86	3-06	0-14	2-86	5-57	67-51	0-172	0-110	10-90	283-5

TABLE Ih.

Condiments, spices, etc.

Name.	Botanical name.	Source.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
1	2	3	4	5	6	7	8	9	10	11	12	13	14
'Arisithippili'	<i>Piper elusii</i>	Local bazaar	12.53	13.24	4.66	6.03	5.17	58.37	0.462	0.278	13.54	328.4	
Asafoetida	<i>Ferula narthex</i>	"	16.03	3.95	1.13	6.99	4.12	67.78	0.692	0.045	22.20	297.1	
Cardamom	<i>Elettaria cardamomum</i>	"	19.95	10.21	2.18	5.37	20.10	42.19	0.128	0.163	5.00	229.2	
Chillies (green)	<i>Capiscum annuum</i>	"	82.60	2.87	0.59	1.04	6.73	6.17	0.029	0.079	1.31	41.5	
" (dry)	"	"	10.02	15.88	6.24	6.10	30.15	31.51	0.163	0.371	2.25	245.7	
Cloves	<i>Eugenia caryophyllata</i>	"	23.29	5.24	8.90	5.20	9.54	47.83	0.739	0.097	4.90	292.4	
Coriander seeds	<i>Coriandrum sativum</i>	"	11.15	14.08	1.04	4.39	32.58	36.76	0.630	0.366	17.94	212.7	
Cumin	<i>Cuminum cyminum</i>	"	11.94	18.71	0.58	5.84	11.98	50.95	1.083	0.487	31.00	283.9	
fenugreek seeds	<i>Trigonella fenugracum</i>	"	13.70	26.19	5.77	3.00	7.16	44.18	0.157	0.365	14.10	333.4	
Garlic	<i>Allium sativum</i>	"	62.79	6.31	0.13	1.02	0.80	28.95	0.025	0.305	1.31	142.2	
Ginger	<i>Zingiber officinale</i>	"	80.86	2.33	0.94	1.19	2.42	12.26	0.016	0.063	2.57	66.8	
'Kandanthippili'	<i>Piper roxburghii</i>	"	12.24	6.44	2.32	4.77	8.51	65.72	1.225	0.188	62.10	309.5	Roots.
Mace	<i>Myristica fragrans</i>	"	15.92	6.51	24.36	1.58	3.76	47.87	0.181	0.103	12.64	436.8	
Mustard	<i>Brassica juncea</i>	"	8.46	22.04	39.64	4.19	1.80	23.87	0.488	0.704	17.88	540.4	
(also referred to under oil-seeds).													
Nutmeg	<i>Myristica fragrans</i>	"	14.32	7.49	36.44	1.72	11.59	28.44	0.122	0.235	4.57	471.7	
Onum	<i>Carum copiticum</i>	"	8.92	15.41	18.08	7.09	11.87	38.63	1.422	0.297	14.62	378.9	
Onion (big)	<i>Allium cepa</i>	"	86.76	1.22	0.04	0.36	..	11.62	0.176	0.046	0.66	51.7	
" (small)	"	"	84.33	1.80	0.08	0.55	..	13.24	0.040	0.058	1.18	60.9	
Pepper (dry)	<i>Piper nigrum</i>	"	12.86	11.53	6.79	4.40	14.92	49.50	0.460	0.199	16.80	305.2	
Tamarind (also referred to under fruits).	<i>Tamarindus indicus</i>	"	20.86	3.06	0.14	2.86	5.57	67.51	0.172	0.110	10.90	283.5	Pulp only.
Turmeric	<i>Curcuma longa</i>	"	13.08	6.33	5.10	3.51	2.60	69.38	0.146	0.284	18.60	348.7	

TABLE II.
Flesh foods.

Name.	2	3	4	5	6	7	8	9	10	11	12
	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Mineral matter per cent.	Fibre per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.	REMARKS.
1											
Beef (muscle)	74.33	22.61	2.58	0.99	..	..	0.004	0.190	0.77	113.7	} Varieties of fish commonly con- sumed on the West Coast.
Crab (")	83.49	8.87	1.07	3.20	..	3.37	1.370	0.147	21.20	58.6	
Egg (duck) ..	71.01	13.54	13.72	1.03	..	0.70	0.073	0.261	2.97	180.4	
" (fowl) ..	73.65	13.33	13.32	0.95	..	..	0.056	0.271	2.07	173.2	
Fish, Mangalore (muscle)	78.38	22.60	0.06	0.83	..	..	0.020	0.185	0.83	90.9	
" " (")	77.89	21.49	1.63	2.03	..	..	0.055	0.413	2.28	100.6	
" Vajra (")	79.41	19.92	1.51	1.38	..	..	0.044	0.379	0.72	93.3	
Liver (sheep)	70.37	19.29	7.49	1.45	..	1.40	0.006	0.378	6.30	150.2	
Mutton (muscle)	71.46	18.54	13.30	1.26	..	..	0.147	0.151	2.52	193.9	
Pork (")	77.41	18.66	4.40	1.01	..	..	0.034	0.198	2.27	114.2	
Prawn (")	77.94	20.84	0.28	1.42	..	..	0.088	0.244	0.79	85.9	

TABLE Ij.
Miscellaneous foodstuffs.

Name.	Moisture per cent.		Protein per cent.		Ether extractives per cent.		Mineral matter per cent.		Fibre per cent.		Carbohydrates per cent.		Calcium (Ca) per cent.		Phosphorus (P) per cent.		Iron (Fe) mg. per cent.		Calorie value per 100 g.		REMARKS.
	1	2	3	4	5	6	7	8	9	10	11	12									
Jaggery or 'ghur' ..		3.93	0.36	0.06	0.62	..	95.03	0.075	0.038	11.40	382.1										
Curds ..		90.31	2.86	2.94	0.59	..	5.30	0.120	0.088	0.31	59.1										
Butter-milk ..		97.54	0.84	1.08	0.14	..	1.40	0.030	0.030	0.78	18.7										
Betel leaves (<i>Piper betle</i>)		85.41	3.07	0.80	2.25	2.33	6.14	0.227	0.044	5.74	44.0										
'Perandai' (<i>Vitis quadrangularis</i>).		87.42	1.21	0.34	2.01	1.83	7.19	0.651	0.050	2.12	36.7										
'Pappads' ..		20.32	18.76	0.33	8.17	..	52.42	0.080	0.298	17.24	287.7										Black gram pre- paration.
Sweet toddy ..		84.72	0.10	0.17	0.66	..	14.35	0.149	0.011	0.26	59.3										
Skim-milk powder ..		4.10	83.04	0.09	6.83	..	50.94	1.370	0.995	1.43	356.7										

Over 200 foodstuffs were analysed by the methods mentioned above. The fractional analysis of the ash, viz., calcium, phosphorus, and iron, was done in duplicate and the results, given in columns 10, 11, and 12, represent the mean of the two results. The results, expressed as a percentage of the original sample, are set forth in the tables (Table I *a* to *j*); Table II summarizes roughly the results for each group of foodstuffs:—

TABLE II.

Showing the average chemical composition of each group of foodstuffs.

Group of foodstuffs.	Moisture per cent.	Protein per cent.	Ether extractives per cent.	Total mineral matter per cent.	Carbohydrates per cent.	Calcium (Ca) per cent.	Phosphorus (P) per cent.	Iron (Fe) mg. per cent.	Calorific value per 100 g.
Cereals	14.53	8.53	1.54	1.78	71.91	0.052	0.224	4.66	335.7
Pulses	11.48	24.47	3.39	3.08	55.58	0.135	0.387	6.92	350.7
Leafy vegetables ..	80.81	4.89	0.85	2.16	10.21	0.334	0.080	11.45	68.1
Root and tuber vegetables.	77.85	1.31	0.14	0.95	19.57	0.054	0.042	0.90	84.8
Other vegetables ..	85.17	2.12	0.25	1.00	9.98	0.058	0.053	2.38	50.7
Nuts and oil-seeds ..	8.96	20.08	46.54	2.69	19.07	0.273	0.448	6.15	575.5
Fruits	79.63	1.28	0.72	0.65	16.50	0.027	0.032	1.26	77.6
Condiments, spices, etc.	29.64	9.56	7.86	3.68	40.13	0.407	0.232	12.51	269.6
Flesh foods ..	75.94	18.15	5.40	1.41	0.50	0.172	0.252	3.87	123.2

DISCUSSION.

The cereals are rich in carbohydrates and fairly rich in proteins, their protein content ranging from 7 to 12 per cent. Their content of fat and calcium is small in comparison with that of certain other kinds of foodstuffs; iron and phosphorus are present in relatively greater amounts. They contain, as a rule, more phosphorus than calcium, just the reverse of green leafy vegetables, which suggests one of the advantages of supplementing a largely cereal diet by vegetables.

Pulses are good sources of protein, containing on an average 24.47 per cent. They are somewhat richer than cereals as regards most chemical constituents, and on the average more than twice as rich in proteins.

The vegetables are, in general, poor sources of the 'proximate principles', but are fairly good sources of the mineral salts. Most of them contain fair amounts of fibre and therefore supply roughage. The leafy vegetables are exceptionally good sources of iron.

The nuts and oil-seeds are naturally rich in fats; they contain in addition fair amounts of protein, calcium, phosphorus, and iron.

The fruits are poor sources of the 'proximate principles' and mineral salts. Their chief dietary value is probably their richness in ascorbic acid or vitamin C.

The condiments and spices are mostly good sources of iron; a few of them are also rich in calcium and phosphorus.

In general, the iron content of the foodstuffs analysed was found to exceed the iron-content figures given in standard textbooks. In this respect our analyses correspond with those carried out by Rosedale (*loc. cit.*) on Malayan foods.

Special mention may be made of a few common and inexpensive foodstuffs, grown and available in abundance in India, which appear, on the basis of chemical analysis, to possess high nutritive value. Thus, amaranth leaves and drumstick leaves, despite their high moisture content—about 80 per cent—are rich in proteins, calcium, phosphorus, and iron. They are very cheap, especially in the rural parts of India. Again, soya bean, a foodstuff that is receiving wide attention in India and elsewhere, is unique among the pulses in containing over 40 per cent of protein. It is also rich in fats and in the three minerals. A pound of it costs about an anna, and the cost per unit of money, of protein and fat, in this form is relatively small.

It must be emphasized that the chemical analysis of foodstuffs, as described in this paper, requires supplementing in certain directions. While it is valuable to know the percentage of proteins a foodstuff contains, it is also necessary to know the biological value of the proteins; similarly the *availability* of mineral salts contained in a food may be as important as their concentration. Further, the analyses were carried out on foods in the state in which they are bought and not in the state in which they are consumed. Investigations are being carried out in the laboratories to throw light on all these points, and to complete the survey in its various aspects.

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THE STATE OF NUTRITION OF SCHOOL CHILDREN IN SOUTH INDIA.

Part II.

DIET AND DEFICIENCY DISEASE IN RESIDENTIAL HOSTELS.

BY

W. R. AYKROYD,

AND

B. G. KRISHNAN.

(Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.)

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IN a previous paper published from these Laboratories (Aykroyd and Rajagopal, 1936) the results of the examination of some 2,000 day-school children in three South Indian towns were given. The objects of the present investigation, in which we were concerned with children in residential hostels, may be described as follows : we wished (*a*) to collect information about institutional diets in South India, (*b*) to study the 'state of nutrition', assessed by various criteria, of children whose diet was known with reasonable accuracy, and (*c*) to compare their condition with that of children in the general population. With regard to (*b*), special attention was given to the incidence of certain symptoms of food deficiency disease—phrynoderma, angular stomatitis, and Bitot's spots—to which reference was made in previous papers (Aykroyd and Krishnan, 1936*b* and *c*; Aykroyd and Rajagopal, *loc. cit.*). We hoped that by observing the incidence of these symptoms in relation to diets of known composition it might be possible to discover the precise deficiencies which give rise to them; another possibility was the discovery of how much vitamin A and carotene in the diet are needed to prevent Bitot's

spots (xerophthalmia). It will be shown how far the original objects of the investigation were attained.

METHOD OF INVESTIGATION.

Residential hostels for children abound in South Indian towns, their usual purpose being to provide a home for country children studying at school. The majority of hostels visited by us are supported by Christian Missions of various denominations; a few are run by Hindu Missions or by the Government. Generally speaking, mission hostels have to feed and lodge children at a very low level of expenditure; government hostels are financed on a slightly more generous scale. The children in Christian Mission hostels belong, for the most part, to the 'depressed classes' and come from rural districts. Children usually remain in the hostels for a number of years, returning home for brief holidays three times a year.

In all we visited 30 hostels. All except two of these were situated in various parts of the Madras Presidency; the two exceptions were located in Mysore. In the final records we have eliminated six hostels, owing, in most cases, either to uncertainty regarding the accuracy of food records obtained or to some other irregularity or drawback (e.g., frequent absences of children which made it difficult to calculate food intake per consumption unit). The investigation, as here presented, includes 24 hostels, housing 719 boys and 955 girls, of ages ranging from 4 to 20 years. The majority fell in the age groups 9 to 17. In each hostel we weighed and measured the children and examined them for phrynoderma, angular stomatitis, and Bitot's spots. Standards of examination were those previously described (Aykroyd and Rajagopal, *loc. cit.*). Particulars about diet were obtained as follows: a single 'typical' month previous to the investigation was selected, and records of expenditure on food during that month were obtained; we found that almost invariably a monthly supply of the main items in the diet was bought at the beginning of each month, leaving little over at the end. Further questions were put to the housekeeper or superintendent to fill in gaps; it was always necessary, for example, to inquire about the supply of fresh vegetables, usually purchased two or three times a week in the local market. It may be objected that any adequate picture of diet throughout the year cannot be obtained from the food intake of a single month, which might be any month of the twelve. But we found that there is very little variation in diet throughout the year in hostels of the type visited. Expenditure on food in most cases was at the level of Rs. 3 to Rs. 4 per head per month, and at this level little variation is possible in the general composition of the diet (e.g., in the quantity of cereal relative to that of other foods). In most cases we were told that the diet schedules had remained the same for some years. We are satisfied that in each case the diet supplied throughout the year *resembled closely* the diet supplied during the month chosen for investigation.

Table I gives the content of the various diets as regards calories, proximate principles, calcium, phosphorus, and iron, with the percentage of total calories derived from cereals. The percentage incidence of phrynoderma, angular stomatitis, and Bitot's spots in the various groups is recorded. In Table II, the foodstuff composition of a few of the diets is given; these may be regarded as typical of the remainder.

TABLE I.
Diet and percentage incidence of deficiency disease symptoms in boys' hostels.

Hostel No. :—	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean.
Number of boys :—	63	32	50	61	51	53	60	21	185	30	113	..	..	65
Protein (g.) ..	96	56	55	79	72	63	72	78	47	79	80	..	..	71
Fat (g.) ..	56	10	10	28	54	33	21	23	15	51	25	..	..	30
Carbohydrates (g.) ..	533	410	458	559	496	459	501	570	404	482	451	..	..	484
Calories ..	3,033	1,945	2,141	2,793	2,763	2,382	2,482	2,713	1,961	2,728	2,352	..	..	2,481
Calcium (g.) ..	0.35	0.16	0.18	0.27	0.79	0.24	1.22	0.35	0.20	0.51	0.28	..	..	0.42
Phosphorus (g.) ..	0.94	0.99	0.97	2.40	1.30	1.04	1.72	2.08	1.05	1.42	1.70	..	..	1.42
Iron (mg.) ..	23.70	19.30	17.40	52.10	31.70	26.10	40.60	22.60	22.00	34.40	42.50	..	..	30.20
Vitamin A (γ) ..	61	25	21	45	232	3	36	14	2	2	2	..	..	..
Carotene (γ) ..	1,815	617	688	1,401	1,830	1,225	2,100	209	515	925	1,138	..	..	..
Percentage calories derived from cereals.	64	86	88	77	64	75	82	83	85	56	82	..	..	..
Percentage incidence of angular stomatitis.	32	50	51	34	4	30	0	25	7	0	13	..	..	..
Percentage incidence of Bitot's spots.	6	3	16	23	4	4	5	0	6	8	7	..	..	..
Percentage incidence of pityrioderma.	11	34	22	53	0	13	9	0	6	4	4	..	..	..

TABLE I—*concl.**Diet and percentage incidence of deficiency disease symptoms in girls' hostels.*

Hostel No.:—	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean.
Number of girls:—	50	58	36	87	66	54	88	69	100	167	39	81	60	74
Protein (g.) ..	63	62	64	52	59	63	87	58	59	54	87	60	67	64
Fat (g.) ..	61	23	13	10	12	25	34	30	16	20	34	13	39	25
Carbohydrates (g.) ..	422	429	475	412	432	386	448	361	411	412	532	503	444	436
Calories ..	2,418	2,167	2,273	1,912	2,073	2,011	2,460	1,948	2,017	2,024	2,788	2,370	2,496	2,221
Calcium (g.) ..	0.72	0.76	0.41	0.54	1.53	0.63	0.80	0.66	0.52	0.31	0.78	0.85	0.54	0.70
Phosphorus (g.) ..	1.25	1.25	1.19	1.14	1.52	1.24	2.19	1.30	1.09	1.09	1.42	1.28	1.34	1.35
Iron (mg.) ..	29.20	33.10	24.50	25.80	35.30	18.70	58.30	35.80	24.70	22.70	22.70	27.60	32.70	30.10
Vitamin A (γ) ..	294	161	2	12	2	26	1	25	35	243	260	2,133	906	..
Carotene (γ) ..	2,733	1,857	1,235	1,141	2,021	2,123	2,702	1,730	1,721	1,354	4,192	985	1,795	..
Percentage calories derived from cereals.	60	70	84	90	88	77	78	82	75	80	66	92	71	..
Percentage incidence of angular stomatitis.	0	0	71	8	0	0	0	3	0	1	0	9	14	..
Percentage incidence of Bitot's spots.	2	0	3	5	11	16	6	1	1	10	0	3	4	..
Percentage incidence of phrynoderma.	4	0	34	33	27	46	6	12	25	5	5	4	0	..

TABLE II.

Foodstuff composition of various hostel diets.
(Oz. per consumption unit per day.)

Boys' HOSTEL.			Girls' HOSTEL.		
No. 6	No. 7.	No. 8	No. 3.	No. 5	No. 6
Milled parboiled rice.	180	Home pounded par-boiled rice	175	Milled parboiled rice.	Milled parboiled rice.
Dhal arhar (<i>Cajanus indicus</i>)	18	Whole wheat	15	Ragi	Ragi
Bengal gram (<i>Cicer arietinum</i>)	01	Ragi	15	Dhal arhar	Cholam (<i>Sorghum andropogon</i>).
Tamarind	06	Dhal arhar	03	Bengal gram	Dhal arhar
Coriander seeds	01	Bengal gram	03	Coriander seeds	Black gram
Onions	03	Green gram (<i>Pisaceo lus mungo</i>)	05	Brinjal	Brinjal
Brinjal (<i>Solanum melongena</i>)	06	Horse gram (<i>Dolichos biflorus</i>)	02	Onions	Onions
Pumpkin	12	Tamarind	01	Potato	Pumpkin
Potato	06	Coriander seeds	03	Plantains	Cucumber
Amaranth (<i>Amaranthus gangeticus</i>)	16	Onions	01	Meat	Green beans
Plantains	02	Bitter gourd (<i>Momordica charantia</i>).	01	Gingelly oil	Potato
Oranges	02	Ridge gourd	01		Drumstick
Meat	02	Cucumber	01		Amaranth
Coco-nut	03	Pumpkin	01		Plantains
Gingelly oil	03	Lady's fingers (<i>Hibiscus esculentus</i>).	01		Nutton
		Green beans	01		Eggs
		Spinach	01		Coco-nut
		Fish	01		Gingelly oil
		Whole milk	02		Jaggery
		Coco-nut	02		
		Coco nut oil	02		
		Jaggery	02		

Intake was calculated per consumption unit ('man value') per day, the earlier International scale of family co-efficients (League of Nations Health Organization, 1932) being employed.

COMPOSITION OF THE DIETS.

The diets as shown in Tables I and II are very typical of those supplied in children's hostels throughout a great part of India, and closely resemble those consumed by the general population. The most common cereal staple used is parboiled milled rice, but millet (usually ragi) is in many instances provided as part of the grain ration. The chief pulse is dhal arhar. Green leafy and other vegetables, and fruit, are provided in relatively small quantities, and as a general rule milk is absent from the diet, or given in very small quantities a few times a week as an addition to tea. A meat meal once a week is often supplied to relieve monotony, and is greatly relished by the children. The chief cereal supplied in hostels usually corresponds to the chief cereal grown in the area in which the hostel is situated.

Average calorie intake in the boys' hostels approximates to the figure which we regard as representing minimum requirements per average man or consumption unit in South India. We were usually informed by hostel superintendents that the children were much more liberally fed in the hostels than in their own homes; entrants are often severely under-nourished and put on a good deal of weight during the months after admission; weight is often lost during the holidays. While it appears that in a number of the hostels the food supply tended to be insufficient in quantity, there can be little doubt that in general the statement of superintendents regarding under-nourishment on admission and subsequent improvement is correct. Diet surveys of poor families of a type similar to those from which the hostel children are drawn would probably reveal a lower calorie intake per consumption unit, and hostel intake is a *better* guide to requirements than intake in the general population. Obviously, however, it is not a completely satisfactory guide, since such institutions are for the most part run at a minimum level of expenditure, and may fail to supply sufficient food fully to satisfy the children's appetites.

Average energy intake per consumption unit was lower in the girls' hostels than in the boys'. Actually we gained the impression that catering arrangements in the girls' hostels were more efficient, the reason being that missionaries and superintendents in charge of girls' hostels are of the female sex. On the whole, the girls were a better nourished group. Theoretically, if food consumption is just adequate in two groups, male and female respectively, calories per consumption unit should work out to the same figure, allowance being made in the scale of consumption co-efficients for lower female requirements. If, in such circumstances, they do not, the scale of co-efficients must be at fault. We are inclined to think that this is the case in the present instance. In the International scale, female consumption is reckoned as 0.8 of male consumption in the age groups over 14, and probably this factor is too high. Mason and Benedict (1931) have shown that basal metabolism of a group of South Indian women was 17 per cent below American standards, while according to Rahman (1936) that of a group of young male students

in Hyderabad was only about 7 per cent below the same standards. Adolescent Indian girls, compared with their brothers, are placid and do not play energetic games. Probably, therefore, a lower fraction of male requirements should be assigned to females over 14. 0.7 may be suggested. If calorie intake in the girls' hostels were worked out on this basis, it would approximate more closely to that calculated in the boys' hostels. The lower calorie intake in the girls' hostels does not justify the assumption that the food of the girls was less adequate in quantity than that of the boys.

Average protein intake per consumption unit was fairly high, above that observed in village-diet inquiries (Aykroyd and Krishnan, 1937). Very little protein of animal origin was consumed. Fat consumption was low and animal fat was lacking. Differences in protein and fat intake in the various hostels were to a considerable extent dependent on the relative quantities of rice and millet consumed. Average calcium intake in the boys' hostels was low, the highest intake being observed in institutions in which ragi formed a considerable part of the diet. In the girls' hostels, ragi was more commonly given, and calcium intake was relatively greater. Phosphorus and iron appear to be present in sufficient amounts in all the diets. The question of vitamin intake will be discussed later in considering the relation between deficiency disease and the quality of the diets.

COST OF DIETS.

In the majority of hostels, cost of food was between Rs. 3 and Rs. 4 per child per month. In a few cases this level of expenditure was exceeded.

HEIGHT AND WEIGHT.

In the majority of hostels we weighed and measured the children ourselves; in some cases, however, we have used records obtained by the superintendent a short time previous to our visit, taking the precaution of checking the accuracy of the scales used against a balance of known accuracy. Originally we intended to compare height and weight in the various hostels as an index of the nutritive value of the diets. This project was, however, abandoned because of the statistical difficulties involved in comparing small numbers belonging to various age groups and also because of lack of knowledge about variation in physique in the parts of the country covered by the inquiry. It is, however, worth while to compare composite height-weight-age averages, based on children in all the hostels, with certain curves based on children of the general population.

Table III gives the average height and weight of hostel boys and girls in the age groups 10 to 17.

In Figs. 1, 2, 3, and 4 these height-weight-age averages are compared with those of day-school children between the ages of 10 and 15 (boys) and 8 and 15 (girls). The day-school boys' measurements were obtained in a previous investigation (Aykroyd and Rajagopal, *loc. cit.*), the children concerned belonging to three South Indian towns. The day-school girls' averages are those published in the Annual Report of the Health Department, Corporation of Madras, for the year 1934; no adequate female height-weight data, relating to the general population,

has as yet been collected by us. The day-school and the hostel groups are roughly comparable as regards the class of children concerned.

TABLE III.

Average height and weight of boys and girls in residential institutions in South India.

Age.	Boys.			Girls.		
	Number weighed and measured.	Height (inches).	Weight (pounds).	Number weighed and measured.	Height (inches).	Weight (pounds).
10	31	51.17	55.02	38	50.23	53.68
11	65	52.06	58.00	82	52.09	57.85
12	98	53.45	60.66	86	53.41	64.88
13	94	56.43	70.09	108	55.82	73.07
14	102	58.83	81.06	75	57.28	80.49
15	73	59.46	81.99	89	58.16	87.95
16	69	61.82	95.25	64	58.40	88.11
17	81	63.35	100.46	46	58.57	89.50

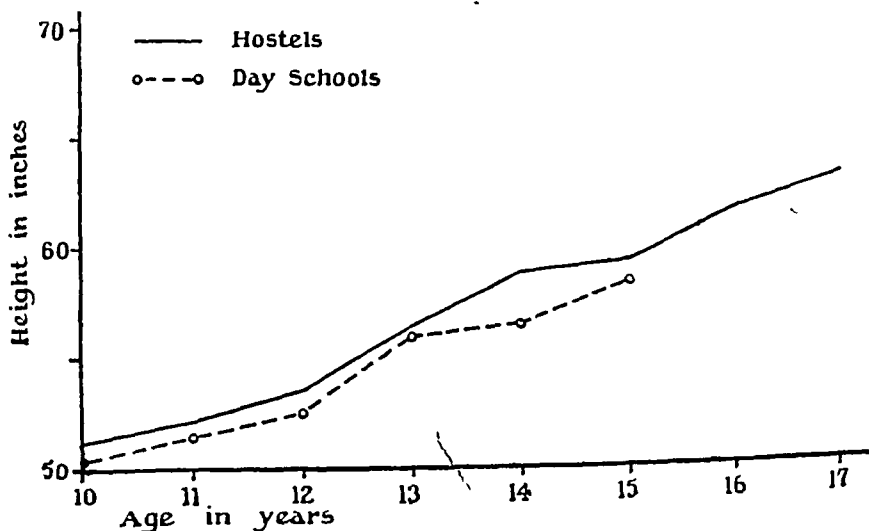


Fig. 1. Height-age averages of boys in hostels and day schools.

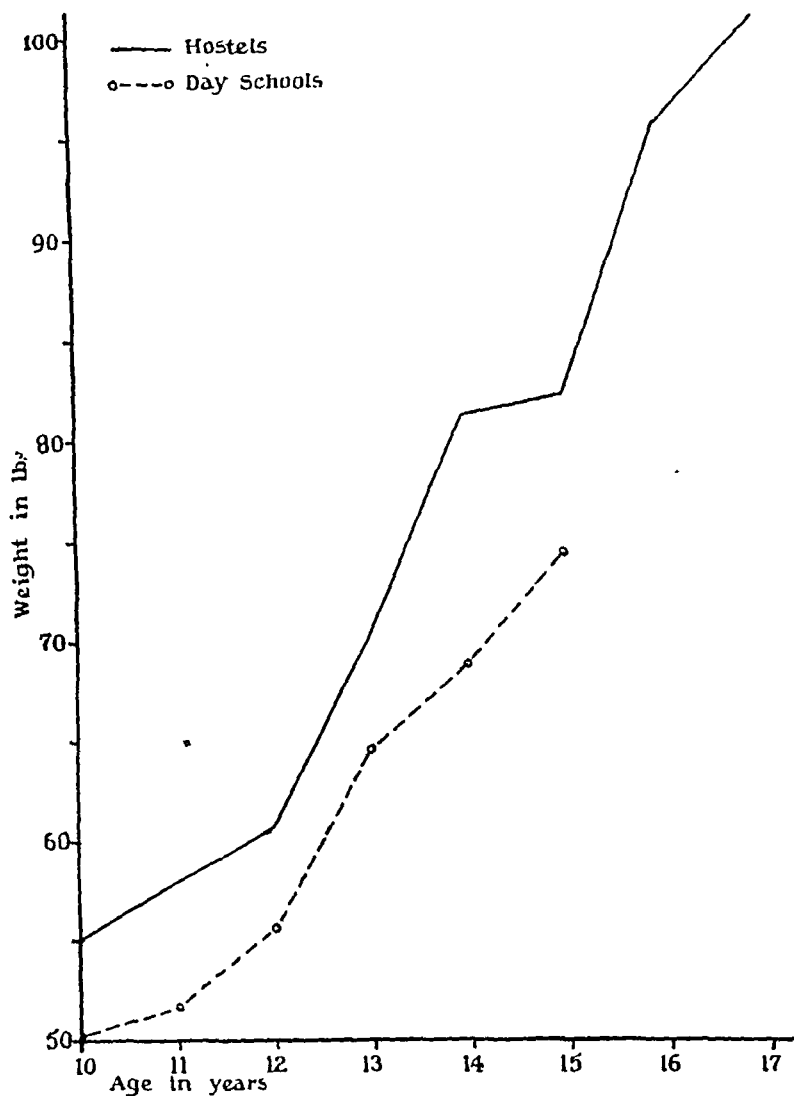


Fig. 2. Weight-age averages of boys in hostels and day schools.

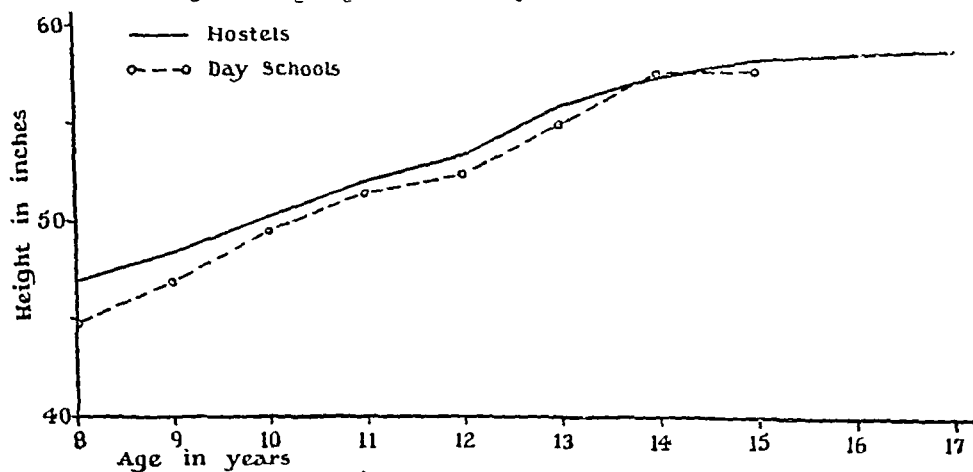


Fig. 3. Height-age averages of girls in hostels and day schools.

Figs. 1 to 4 show that both the height and weight averages of the hostel children are above those of day-school children, the advantage being greater as regards

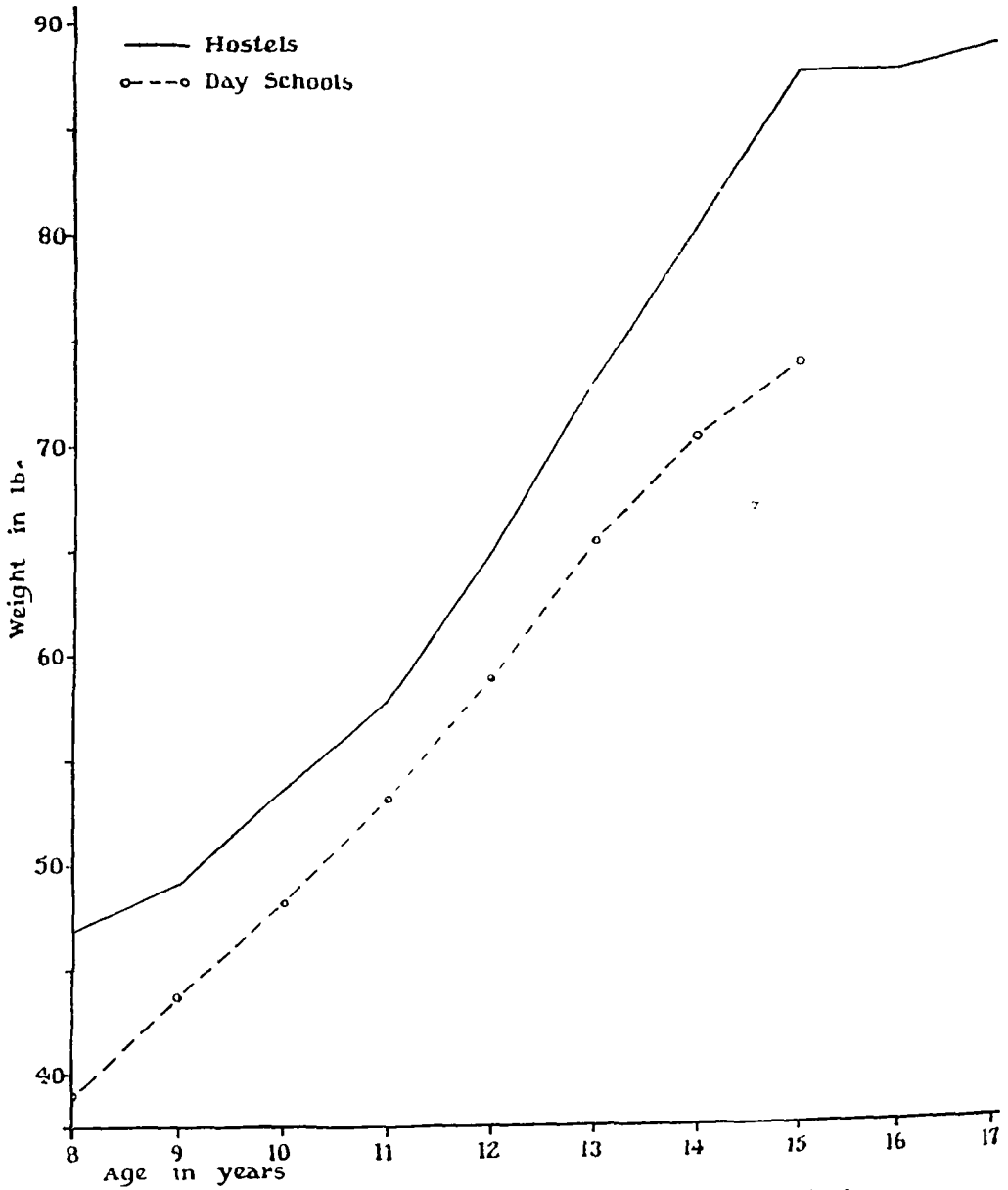


Fig. 4. Weight-age averages of girls in hostels and day schools.

weight. To demonstrate the latter advantage more clearly, curves (Figs. 5 and 6) of average weight on average height in the age groups 10 to 15 of hostel and

day-school children have been plotted. It will be observed that at every age the weight for height of the hostel children is greater than that of the day-school children. This difference is, in our opinion, an expression of the better 'state of nutrition' of the children living in institutions. Their diet may in many instances be poor in quality, but at least they regularly consume three meals per day,

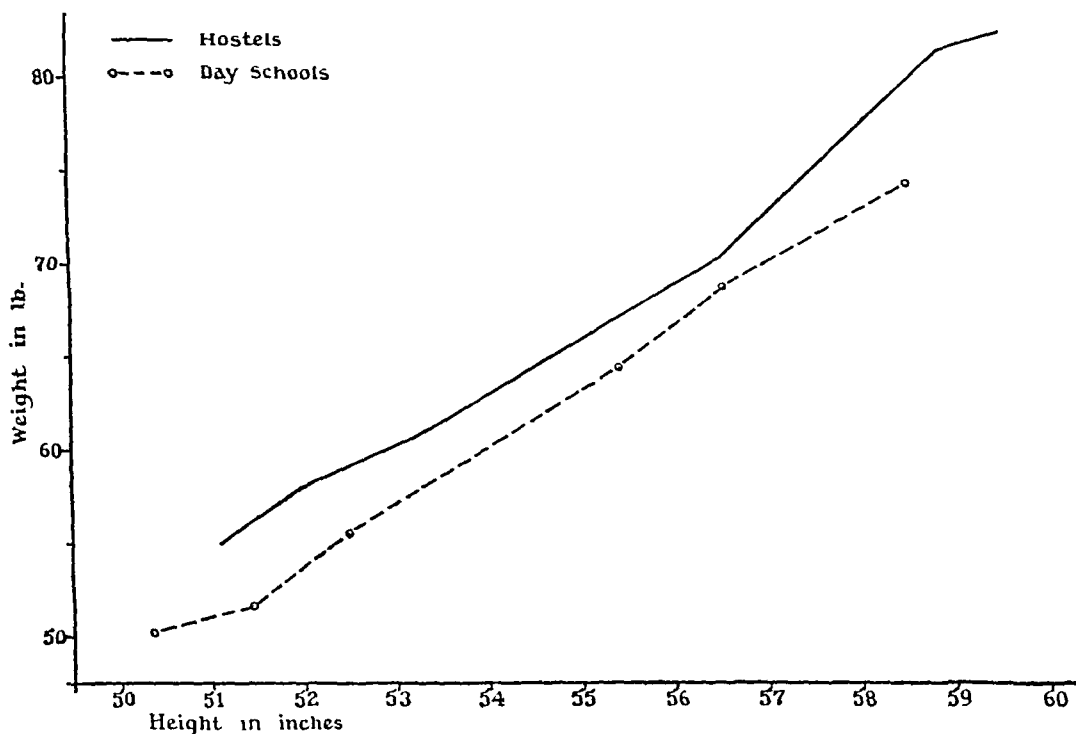


Fig. 5. Weight for height of hostel and day-school boys compared.

while children of the poorer classes in the general population may be less fortunate in this respect. The fact that day-school children are on the average thinner than children fed on diets costing Rs. 3 to Rs. 4 per month throws a light on the diet and economic conditions of the former.

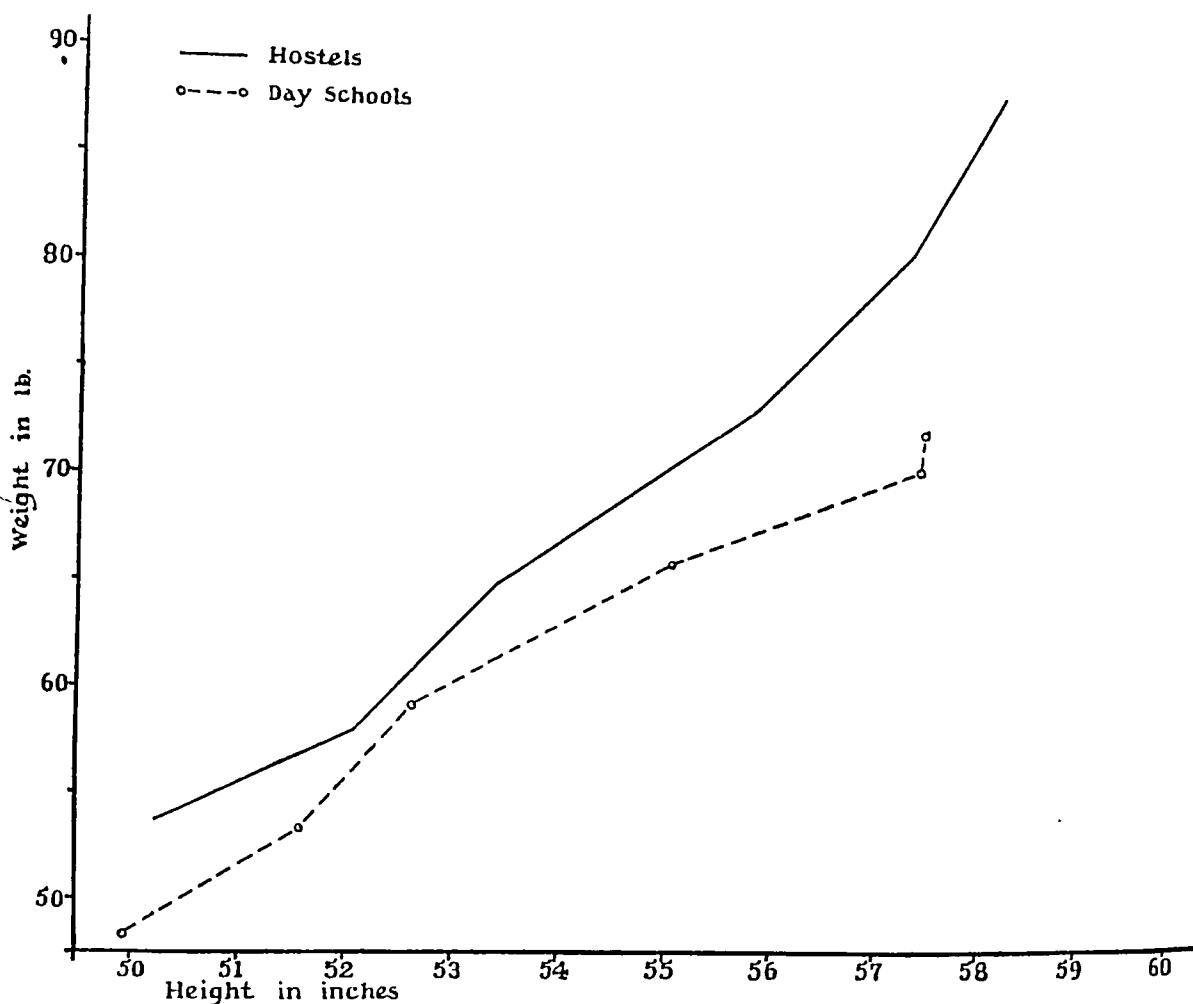


Fig. 6. Weight for height of hostel and day-school girls compared.

THE INCIDENCE OF SYMPTOMS OF FOOD DEFICIENCY DISEASE.

In a previous investigation (Aykroyd and Rajagopal, *loc. cit.*) some 2,000 day-school children in three South Indian towns were examined for angular stomatitis, phrynoderma, and Bitot's spots. It was observed that these symptoms were frequently present. The methods of examination followed here were similar to those previously described. We found that in general these conditions are common in children living in institutions. In recording the percentage incidence, we disregarded children who had been resident for less than 6 months.

Angular stomatitis.—This condition has been fully described in a previous paper (Aykroyd and Krishnan, 1936c). It was pointed out that the characteristic fissures at the angles of the mouth are usually found in association with lesions of the tongue, and that for the systematic examination of large numbers, angular stomatitis is convenient. Both glossitis and angular stomatitis disappear simultaneously on treatment with dried yeast, unheated or autoclaved, and skim-milk. Deficiency of some part of the vitamin-B₂ complex appears to be the causative factor.

As the present investigation proceeded we became aware of a relationship between the nature of the diet and the incidence of angular stomatitis. The condition was most prevalent in hostels in which the bulk of the diet was composed of milled rice, raw and parboiled, and the supply of milk and vegetables was small. In a few hostels, a relatively high consumption of rice was not associated with a high incidence of stomatitis; in these the rice was supplemented by reasonable quantities of milk, vegetables, or (in one case) liver. Where the staple cereal was millet, or where rice and millet were both provided, the latter in fair quantities, angular stomatitis was relatively infrequent. In practice we found that, having examined the children and noted the incidence of stomatitis, we could 'predict' with fair accuracy the nature of the diet. Conversely, we were prepared to wager that cases of stomatitis would be found in any hostel in which an insufficiently supplemented rice diet was supplied.

In Table IV, an attempt is made to demonstrate the relation between stomatitis and the composition of the diet. Hostels are listed in descending order as regards the incidence of stomatitis. The percentage of total calories obtained from rice and the milk intake are given in adjacent columns. Vegetable intake is roughly indicated as above or below 5 oz.

TABLE IV. .

Diet and angular stomatitis.

Number of hostel.	Percentage incidence of angular stomatitis.	Percentage of total calories from milled rice.	Milk intake per consumption unit per day (oz.).	Vegetable intake (above or below 5 oz. per consumption unit per day).	REMARKS.
G 3 ..	71	76	None	Below	0.3 oz. liver per consumption unit per day.
B 3 ..	51	86	Butter-milk, 1.3	Above	
B 2 ..	50	78	None	Below	
B 4 ..	34	77	Butter-milk, 1.0	Below	
B 1 ..	32	62	Whole milk, 1.3	Below	
B 6 ..	30	75	None	Above	
B 8 ..	25	83	Butter-milk, 3.8	Below	
G 13 ..	14	49	None	Above	
B 11 ..	13	42	None	Below	
G 12 ..	9	57	None	Below	
					0.75 oz. liver per consumption unit per day.

TABLE IV—*concl'd.*

Number of hostel.	Percentage incidence of angular stomatitis.	Percentage of total calories from milled rice.	Milk intake per consumption unit per day (oz.).	Vegetable intake (above or below 5 oz. per consumption unit per day).	REMARKS.
G 4 ..	8	60	Curds, 0.6	Below	Very high pulse intake.
B 9 ..	7	69	None	Below	
B 5 ..	4	55	Whole milk, 4.6	Above	
G 8 ..	3	23	Whole milk, 1.2	Below	
G 10 ..	1	69	Whole milk, 0.3	Above	
B 7 ..	0	43	Whole milk, 0.6	Above	
B 10 ..	0	56	Butter-milk, 0.2	Below	
G 1 ..	0	50	Whole milk, 6.4	Above	
G 2 ..	0	62	Whole milk, 5.8	Above	
G 5 ..	0	31	None	Below	
G 6 ..	0	33	None	Below	
G 7 ..	0	7	None	Above	
G 9 ..	0	64	Whole milk, 1.8	Above	
G 11 ..	0	20	Whole milk, 6.7	Above	

In the first seven hostels listed, in which the greatest incidence of stomatitis was found, the percentage intake of rice was high, while milk and vegetable consumption was low. It must be observed that 'butter-milk' in India is often a well-watered product of dubious nutritive value. In the group of hostels in which stomatitis was infrequent or absent, we found in general either a low intake of rice, one of the millets being the staple cereal, or a relatively higher intake of rice combined with a fair intake of milk, vegetables, or other nutritious foods. Attention may be drawn to certain hostels. We have previously demonstrated (Aykroyd and Krishnan, 1936c) the curative effect of skim-milk on stomatitis and the value of liver has been observed by Landor and Pallister (1935); according to these workers, stomatitis in prisoners was cured by giving 8.0 oz. of fresh liver daily. In B 10, G 2, and G 9 there was no stomatitis in spite of a relatively high rice intake; in these hostels there was a fair provision of milk or vegetables, or, in one instance, the consumption of pulses was exceptionally high. In G 5 and G 6 there was no stomatitis, in spite of the absence of milk and a low intake of vegetables. The chief cereal consumed in these hostels was millet (ragi in the former; ragi and cholam in the latter), which formed the bulk of the diet. In G 7 also the principal cereal was millet.

It is not to be expected that all the hostels should fit exactly into the scheme; apart from inevitable inaccuracies in the collection of food consumption data, there are many variables which have not been considered, e.g., intake of pulses and other foods, the varying composition of the vegetable ration as regards leafy and other vegetables, and the varying age composition of the hostel groups. For example, in B 9 there was a relatively high rice intake, no milk, and a poor vegetable supply; angular stomatitis was present, but one would have anticipated a higher percentage incidence. But our observations tend to the conclusion that angular stomatitis is commonly associated with the consumption of a diet largely composed of rice, and deficient in protective foods.

If G 3 and B 2 were to be visited at the time of writing, no stomatitis would be found. In G 3 the condition has disappeared as the result of the addition of 1.5 oz. of skim-milk powder to the original diet, while in B 2 some cases have been cured by unheated and autoclaved yeast and the remainder by a daily intake of 1.0 oz. of skim-milk powder (Aykroyd and Krishnan, 1936c).

Present knowledge of the distribution in foodstuffs of what may be called the 'anti-stomatitis' vitamin can be summarized as follows:—

Milled rice	..	..	..	Absent.
Millet	..	..	..	Present.
Milk, including skim-milk	..	..	..	Abundantly present.
Yeast (unheated)	..	..	..	" "
Yeast (autoclaved)	..	..	..	" "
Liver	..	..	..	" "

PHRYNODERMA.

The literature relating to this condition has been summarized by Radhakrishna Rao (1937). A number of workers support the view that it is due to vitamin-A deficiency. In the present investigation we have been unable to detect any specific relation between the frequency of phrynoderma and the composition of the diet, beyond the fact that in general it was most common in the hostels in which the diet was most deficient in protective foods. The figures of carotene and vitamin-A intake are based on the spectrographic assay of particular samples of each foodstuff. The carotene intake estimates are very rough, since the richest source of carotene was green leafy vegetables, which is just the food the intake of which was most difficult to estimate accurately and varies most from season to season. Further the carotene content of any vegetable foodstuff may fluctuate within wide limits, depending on the period of storage, etc. (De, 1936).

Our observations are not incompatible with the theory that vitamin-A deficiency is one of the causative factors of phrynoderma, but the irregularity of its appearance in groups of malnourished children suggests that some other factors may play a part. In B 8, B 9, and B 10, intake of vitamin A and carotene was low, while phrynoderma was absent in B 8, and present in only a small percentage of boys in B 9 and B 10.

Angular stomatitis and phrynoderma are not caused by the same dietary deficiencies. The incidence of the former, due to deficiency of some factor or factors in the vitamin-B₂ complex, showed no correspondence with that of the latter.

BITOT'S SPOTS.

Bitot's spots is the name usually given to the patches of foamy yellowish-white exudate, appearing on an area of conjunctival xerosis. It was stated at the beginning that we hoped to be able to correlate the frequency of Bitot's spots and the vitamin-A activity of the diet, and hence to throw light on the problem of minimum human requirements of vitamin A and carotene. This expectation has not been fulfilled. A few cases of Bitot's spots were found in nearly all the hostels, but there was no relation between vitamin-A activity as estimated on the basis of spectrographic assays, and the incidence of this eye lesion. As the result of a number of scattered observations we incline to the view that Bitot's spots are a chronic condition which does not readily disappear unless some rich source of vitamin A (e.g., cod-liver oil) is given regularly for some time. Possibly the children with Bitot's spots at the period of inspection showed the same lesion on admission to the institution, resulting from deficient feeding in the earlier years of childhood, and the average hostel diet was not sufficiently rich in vitamin A to cause its disappearance. It is noteworthy that B 4, in which the highest incidence of Bitot's spots was found, is largely a home for destitute orphans. Lieut.-Colonel R. E. Wright, C.I.E., I.M.S., of the Government Ophthalmic Hospital, Madras, has seen occasional cases of Bitot's spots in adults which persist in spite of the consumption of a good diet for many years. More satisfactory evidence about minimum vitamin-A and carotene requirements would be obtained if an *outbreak* of night-blindness and xerophthalmia in an institution could be observed and related to the vitamin-A activity of the diet. The detection of very early commencing signs of xerophthalmia and night-blindness would be of more value, for the establishment of minimum vitamin-A requirements, than the detection of the more chronic condition. The former would require considerable ophthalmological experience in differential diagnosis; Bitot's spots can easily be observed by non-ophthalmologists and we thought it wiser to confine our investigations to a sign in the recording of which there were fewer possibilities of error. We did not study the incidence of night-blindness because of the difficulty of obtaining a satisfactory account from children about a subjective symptom.

In the majority of hostels in which Bitot's spots were observed, vitamin-A and carotene intake was below 2,000 International Units. One γ of carotene and 1 γ of vitamin A, as estimated by De's (1937) method, are roughly equivalent to 1.0 and 2.6 International Units respectively. In the hostel in which the vitamin-A activity of the diet was estimated at the lowest figure (B 8), no cases of Bitot's spots were observed; this was a small hostel in which the boys were of a higher average age than the general average of the hostels. In G 1, G 12, and G 13 a few cases of xerophthalmia were present in spite of an estimated intake exceeding 2,500 International Units. In the two latter hostels the relatively high intake was due to the inclusion of sheep's liver in the diet, one or two meals per week being given in lieu of meat. Two samples of *uncooked* liver investigated in these Laboratories have been found to contain about 100 γ of vitamin A per gramme. It is to be observed that even 6,500 International Units, roughly the estimated intake in G 12, are less than the quantity of vitamin A which would be provided by 0.5 oz. (15 g.) of average cod-liver oil taken daily. We have observed the improvement of a case of chronic xerophthalmia with Bitot's spots which was given roughly this amount of cod-liver oil for 2 to 3 months, as an addition to a

diet of unknown vitamin-A activity. There is an obvious difference between consuming vitamin A provided in the form of steady daily dose of cod-liver oil and provided in the form of one or two meals of cooked liver per week.

We make no attempt to draw from these investigations deductions about minimum or optimum vitamin-A requirements which could be applied in establishing a standard for purposes of practical dietetics. We simply record the persistence of cases of Bitot's spots on diets which appear, in general, to be of low vitamin-A and carotene content, and particularly deficient in vitamin A. The possible errors of the estimates given must not be minimized.

DISCUSSION.

A few remarks on certain practical aspects of these investigations will not be out of place. In return for co-operation on the part of the superintendents of the various hostels, we gave advice about the improvement of diet schedules. Here we were faced with the main problem of practical nutrition work in India; how to make 1 anna do the work of 3 annas. Most of the hostels could afford to increase expenditure on food very little or not at all; it was, therefore, in most cases useless to recommend any of the 'cheap balanced diets' previously described (Aykroyd and Krishnan, 1936*a*) which cost about Rs. 5 per month for an adult and very little less for growing children. To draw up a diet schedule costing Rs. 3-8 per child per month which is thoroughly satisfactory in the light of modern knowledge is impossible. It would have been quite useless in most cases, for example, to offer the recommendation that 8·0 oz. of whole milk should be added to the diet.

There are, however, certain improvements which can be made without great increase in cost. Many of the diets were low in fat; the addition of extra vegetable oil or coco-nut at the expense of a quantity of cereal supplying an equivalent number of calories, does not greatly put up expenditure. (Ghee or butter would be out of the question.)

If the cereal consumed is milled rice, an improvement in the nutritive value of the diet can be brought about by wholly or partially substituting whole rice, whole wheat, or one of the millets; this change involves little addition in cost. It is unfortunate that there is a social prejudice against ragi in South India; it is often regarded as the food of the poor villager—also as the food of prisoners in gaols—and adolescent country boys and girls, who are hoping to raise themselves above the status of their parents, feel entitled to consume the cereal of the townsman and the educated classes—milled rice. Many of the hostel superintendents told us that they had the greatest difficulty in persuading the children to eat ragi and other kinds of millet, even when millet is the staple food of the district from which they come. A further difficulty lies in the fact that ragi is often prepared in an unpalatable form. We have nevertheless succeeded, in some cases, in increasing considerably the millet content of the diet at the expense of rice, with definite improvement in the well-being of the children.

The pulses are relatively inexpensive—in many instances we recommended an increase in intake of 1 oz. to 2 oz. per child per day. Soya bean in small quantities has been introduced into the schedules of one or two hostels.

The consumption of green leafy vegetables can be encouraged. The cheaper varieties—amaranth leaves, coriander leaves, drumstick leaves, etc.—are as nutritious as the more expensive ones, such as lettuce. Here and there, where circumstances were favourable, we suggested the creation of a vegetable garden, to be tended by the children themselves. If advantage is taken of seasonal cheapness, some fruit can usually be supplied with fair regularity at no great expense.

Even a little milk is better than none. An adequate supply of whole milk may be impossible, but a little butter-milk or skim-milk will enhance the nutritive value of hostel diets. In certain institutions we have introduced, with enormous benefit to the children, fairly large quantities of skim-milk reconstituted from New Zealand skim-milk powder, which costs roughly one-third the price of a similar quantity of fresh whole milk. Special mention may be made of one large children's institution, not included in this investigation, in which intake of milk was increased by the creation of a cheap and regular local supply, i.e., by ensuring local producers of a regular market. This institution was, however, in a rather better financial position than the majority, and situated in a district favourable for milk production.

There is a large field for useful work in improving diets in children's residential institutions in India. While the problem of malnutrition among day-school children is very difficult to attack, there is no reason why children in hostels should not receive a moderately satisfactory diet.

SUMMARY.

(1) The composition of the diets of 24 residential children's hostels in South India has been studied. The diets supplied in such institutions, while in general of low nutritive value, are superior to those consumed by the general population.

(2) Children in hostels are superior in weight for a given height to children of the same class in the general population.

(3) Angular stomatitis, phrynoderma, and xerophthalmia were found to be common. The highest incidence of angular stomatitis was observed in hostels in which the diet was largely composed of rice and lacked 'protective' foods. Present knowledge about the distribution in foodstuffs of the 'anti-stomatitis' vitamin is summarized.

(4) No specific relation was observed between the frequency of phrynoderma and the composition of the diet, beyond the fact that in general it was most common in hostels in which the diet was most deficient in protective foods. It is difficult to explain the incidence of this condition on the hypothesis that it is due *solely* to vitamin-A deficiency.

(5) Cases of Bitot's spots were found in the majority of hostels. In general, the diets were low in vitamin-A activity, but no precise relation appeared to exist between the vitamin-A activity of the diets and the frequency of Bitot's spots. The significance of this condition as evidence of malnutrition is discussed.

(6) Useful practical work can be done in improving diets in children's residential institutions. While it is often impossible, with available financial resources, to introduce a really satisfactory diet schedule, various improvements can be made with little increase in cost.

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'PHRYNODERMA'—A CLINICAL AND HISTO-PATHOLOGICAL STUDY.

· BY

M. V. RADHAKRISHNA RAO.

(Nutrition Research Laboratories, I. R. F. A., Coonoor, S. Indis.)

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DURING recent years increasing attention has been paid to the study of the cutaneous manifestations in food deficiency disease. A peculiar dry, scaly consistency of the skin has been described in association with the ocular symptoms by most of the writers on keratomalacia. A detailed clinical description of the changes in the skin in cases of keratomalacia in adults was given by Pillar (1929a and b), who emphasized that keratomalacia is a 'system disease' of the ectodermal tissues, manifesting symptoms not only in the eye but in many parts of the body. Infections of the skin, such as pustular eruptions, furunculosis, abscesses, impetigo, acute bed-sores (in babies), etc., have often been noted in association with keratomalacia. In summarizing the available literature on the subject, Mackay (1934) observed that 'there is no one type of skin infection particularly characteristic of vitamin-A deficiency, but that a variety of skin infections, often chronic or relapsing in character, may occur'.

Besides the above-mentioned changes, a follicular keratosis of the skin has been described occurring in association with xerophthalmia and keratomalacia. Frazier and Hu (1930, 1931) reported the occurrence of spinous papules at the sites of the hair follicles, mostly on the extensor surfaces of the extremities, shoulders, and lower part of the abdomen, in association with a dry and sometimes dark skin, in a group of Chinese soldiers (in a Military Camp near Peiping), who showed typical signs of keratomalacia but no signs of other vitamin deficiency disease. Histologically, the pathological process was one of 'hyperplasia and hyper-keratinization of the epithelium of the epidermis and hair follicles, with associated metaplasia of the glandular structures of the skin and infection'. As a result of providing a well-balanced diet and administering 30 c.c. of cod-liver oil daily, and without any local medication, the papular lesions slowly disappeared leaving delicate atrophic pigmented scars. On the analogy of the pathological changes occurring in the other organs in human and experimental avitaminosis-A, and from the results of the therapeutic tests, these authors concluded that the condition was attributable to a deficiency of vitamin A in the diet. Further clinical and pathological observations on the part of these workers (Frazier and Hu, 1934, 1936) tend to confirm

their original observations on the specific nature of the follicular hyper-keratosis. They have further pointed out that the follicular lesions were most common in young adults and may occur in the absence of the classic ocular signs of vitamin-A deficiency.

A dermatosis, similar to that mentioned above, was described by Loewenthal (1933*a* and *b*) in East Africa. During an inspection of the Uganda Central Prisons, it was found that the majority of a group of prisoners suffering from night-blindness and xerophthalmia also had the cutaneous eruption. Treatment with one ounce of cod-liver oil a day, and without any modification of the diet, for a period of nine weeks, resulted in the cure of night-blindness and xerophthalmia in every case and of the dermatosis in about 98 per cent of cases. The condition was regarded as a dyskeratosis, with changes consequent on this condition; no signs of sepsis or softening were noticed. It was also pointed out that the histological changes were precisely the same as those occurring in the papules of *Pityriasis rubra pilaris*, although the anatomical distribution of the two conditions was different. The dermatosis was attributed to a deficiency of vitamin A in the diet. The papular eruption was later taken as one of the criteria of vitamin-A deficiency in the inspection of schools (Loewenthal, 1935*a*) and was subsequently included under the accepted signs and symptoms of vitamin-A deficiency (Loewenthal, 1935*b*).

Similar, but independent, observations were made by Nicholls (1933) on the occurrence of a papular dry skin, which was frequently accompanied by mild neuritis, keratomalacia or diarrhoea, among poorly fed labourers in East Africa and also among convicts in a jail in Ceylon. He gave the condition the name of 'phrynoderma' (toad-skin) (Nicholls, 1933, 1934, 1935 and 1936). He attributed it primarily to a vitamin-A deficiency in the diet, but considered that deficiency of other food factors may also be responsible. He further pointed out that phrynoderma, which is a sign of dietary deficiency, is of great value in determining the 'state of nutrition' of groups of persons (Nicholls, 1935).

Goodwin (1934) in London, and Sweet and K'ang (1935) in Peiping (China), described similar follicular keratotic lesions in association with vitamin-A deficiency. Wright (1936) in Madras (India) described papular skin lesions similar to those of *Pityriasis rubra pilaris*, occurring on elbows and knees, both in adults and children suffering from keratomalacia. Giblin (1936) has long observed cutaneous manifestations, similar to those described by Loewenthal (1933*b*), associated in some cases with xerophthalmia, in the syndrome of vitamin-A deficiency in Papuan natives. Wright, E. J. (1930), describing 'A and B avitaminosis disease' in Sierra Leone, mentioned a keratosis of the skin of the limbs and trunk, the skin becoming dry and rough.

The incidence of phrynoderma in groups of school children in South India has recently been reported from these Laboratories by Aykroyd and Rajagopal (1936). In this paper, the clinical manifestations and the histo-pathological features of the condition have been described and its ætiology is discussed.

CLINICAL OBSERVATIONS.

The clinical observations described in this paper were made on children attending the local schools and the Municipal Child-Welfare Centre, and on adults and children in the Government Lawley Hospital, Coonoor. Their ages varied

from 3 to 35 years. Most of them belonged to the poorer classes and the diet which they obtain in their homes usually consists mainly of milled rice, a small quantity of various kinds of pulses and vegetables with condiments and a little millet; 'protective' foods such as milk, milk products, meat, eggs, etc., are consumed only in very small quantities occasionally or not consumed at all.

The general condition of the individuals showing phrynoderma, taking the group as a whole, was poor. A 'chubby' child was, however, occasionally seen with no other signs of ill health apart from the cutaneous manifestations. In some instances, especially in children, phrynoderma was associated with 'angular stomatitis'. The association of typical 'Bitot's spots' is not common, but varying degrees of irregular xerosis, loss of lustre, discoloration, or in some instances wrinkling, of the conjunctiva, were often present. The skin and, in most cases, the hair were generally dry.

The papular eruption.

(a) *Onset*.—In most instances the onset was insidious, some of the patients being unaware of its existence till the time of examination. A dry and slightly rough skin was sometimes noticed preceding the appearance of the typical papules.

(b) *Subjective symptoms*.—Subjective symptoms were absent and, even in response to leading questions, no pain or itching was complained of, and there was no tenderness.

(c) *Site*.—The papules were situated at the site of the pilo-sebaceous follicles, and microscopic examination revealed that the lesions were mainly in and around the latter.

(d) *Distribution*.—In most instances the papules were roughly symmetrical in their distribution and were either profuse or localized. They were generally discrete and tend to appear in groups. The papules were present mostly on the extensor surfaces of the arms and thighs (see Plate XXVI) and the upper part (postero-lateral aspect) of the forearms near the elbows; in some they were also present on the extensor surface of the upper part of the legs, posterior axillary folds, over and in between the scapular regions, and across the buttocks and flanks. Acne-like eruptions on the face were not a conspicuous feature, although the skin in this situation was also dry as in the other areas. The genito-anal regions and the hands and feet were not affected by the papular eruption.

(e) *Description of the papules*.—The papules varied in size from a big pin's head to a millet seed, and were rounded or hemispherical in outline with sharply defined edges (see Plate XXVI). Their surface was slightly rough and presented a central keratotic plug, which either projected from the atrophied hair follicle or was surmounted by a loosely adherent scale. The colour of the papules resembled that of the skin but, in some instances, hyper-pigmentation was evident in, and immediately around, the papules. The papules were firm in consistency; in some children in whom the papular eruption resembled an exaggerated and permanent 'goose skin' (*Cutis anserina*), the papules were not hard as in the others.

(f) *Clinical examination*.—Phrynoderma was present in children of both sexes as well as in adults. To the touch the affected areas gave a sensation of passing over a nutmeg grater surface. Sensation in response to light touch, pin-prick,

and heat and cold was apparently normal in the areas of skin affected by the eruption.

(g) *Course and evolution*.—The papules showed no tendency to pustulation. In one case—a boy aged 2½ years with spinous papules in most of the situations described above—which improved under treatment with cod-liver oil (half an ounce twice daily) and a well-balanced diet for a period of about two months, small delicate cicatrices were noticed in areas from which the papules had disappeared. It is hoped to undertake further therapeutic tests on cases of phrynoderma and observe its course under treatment.

HISTO-PATHOLOGICAL FEATURES.

Material for histo-pathological study has been obtained by biopsy from ten hospital patients, showing typical papules. Serial sections of the skin were cut in each instance and stained by Ehrlich's acid-hæmatoxylin and eosin, Weigert's iron-hæmatoxylin and van Gieson's stain and orcein.

(i) *Epidermis*.—There was a superficial hyper-keratosis of the epidermis in all cases; in places, the *stratum corneum* was very much broadened (see Plate XXVII, figs. 3 and 4). The keratin was either homogeneous in structure or present in loose meshes. There was no evidence of para-keratosis.

No appreciable changes were present in the *stratum lucidum* and the *stratum granulosum*.

The *rete mucosum* in some places, especially in the neighbourhood of the atrophied hair follicles, showed moderate hypertrophy (see Plate XXVII, figs. 5 and 6). The interpapillary processes were widened and more prominent in these areas. The prickle cells appeared normal but, in places, showed vacuolation in their protoplasm.

The cells of the *stratum germinativum* were apparently normal.

Evidence of hyper-pigmentation was not marked even in the neighbourhood of the papules; in some places, however, the pigment-bearing cells were increased in number and were distributed throughout the thickness of the *rete mucosum*.

(ii) *Corium (cutis vera)*.—But for some of the changes described below in the perifollicular regions, the corium (*cutis vera*) was apparently normal. In three cases, however, there was slight perivascular round-celled infiltration. Otherwise there was no congestion, no cedema or hæmorrhages. The fibrous and elastic tissues (see Plate XXIX, fig. 11) appeared normal. Very few chromatophores were present in the superficial layers of the corium.

(iii) *Sebaceous glands*.—The sebaceous glands were not seen in connection with the atrophied hair follicles (see Plate XXVIII, fig. 10; Plate XXX, figs. 15, 16, and 18). Serial sections, however, revealed that the glands were still present in some areas in which the follicular lesions were not very marked; in these areas there was a varying degree of hyper-keratinization of the lining epithelium of the funnels of the hair follicles, and the sebaceous glands showed either a decrease in the amount of cytoplasm or in the number of cells (see Plate XXVIII, figs. 7 to 10). At a later stage of the follicular lesion, a few cells with very little cytoplasm represented the glands; in the typical papules they were atrophied and their site was generally occupied by young connective tissue and mononuclear lymphoid cells.

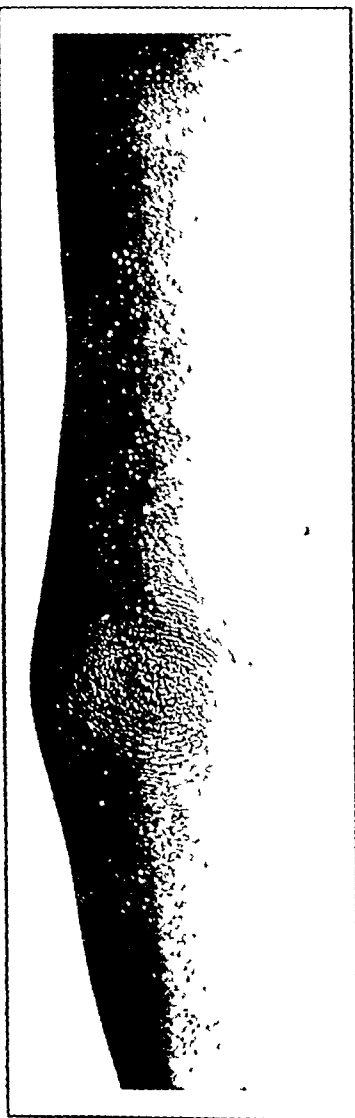


Fig. 1.



Fig. 2.

Figs. 1 and 2. Photographs showing the papular cutaneous lesions on the extensor aspects of the limbs in a boy aged 11 years. Note the ichthyotic dry skin on the leg.

[All the photomicrographs were taken with 'Miflex' (Zeiss)].



Fig. 3.



Fig. 4.

and 4. Superficial hyper-keratosis of the epidermis; note the broadening of the *stratum corneum*. An atrophic hyper-keratotic hair follicle is seen on the right in Fig. 4. $\times 50$.



Fig. 5.

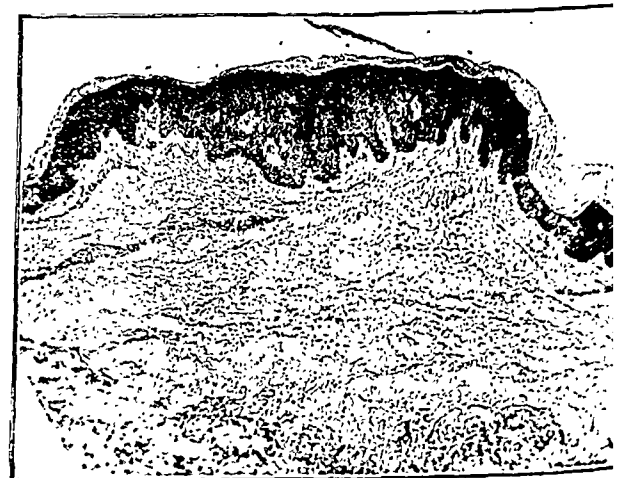


Fig. 6.

Figs. 5 and 6. Epidermis showing epithelial hyperplasia. $\times 50$.

PLATE XXVIII.



Fig. 7.



Fig. 8.



Fig. 9.



Fig. 10.

Figs 7 to 10. The lining epithelium of the funnels of the hair follicles shows varying degrees of hyper-keratinization; the sebaceous glands, which are still present, show a decrease in the amount of cytoplasm or in the number of cells (the sebaceous gland in Fig. 7 is almost normal). An advanced stage of follicular lesion is seen on the left in Fig. 10. (Fig. 10. Weigert's iron-haematoxylin and van Gieson's stain). $\times 50$.



Fig. 12.

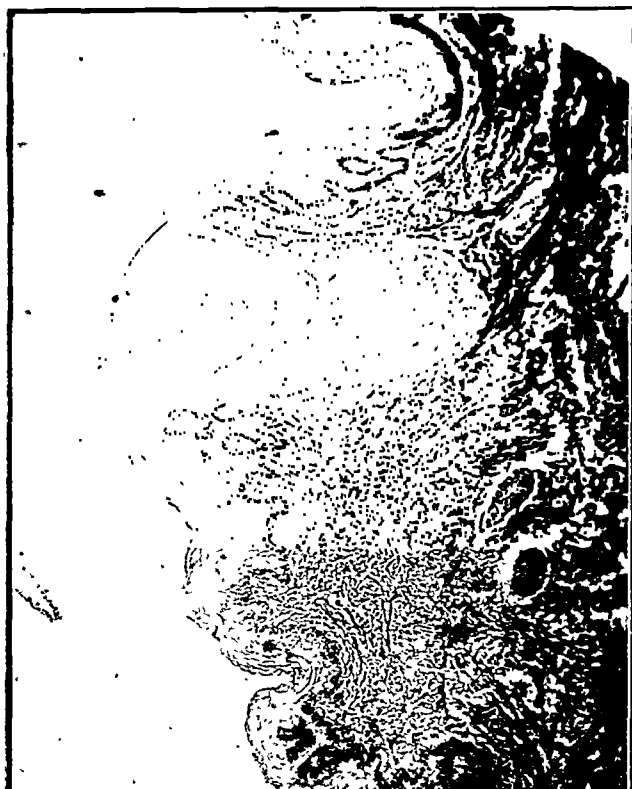


Fig. 11.



EXPLANATION OF PLATE XXIX.

- Fig. 11. Section of a papule showing the normal distribution of elastic tissue in the corium. The elastic fibres are stained dark; Orcein. $\times 50$.
- „ 12. Group of sweat glands showing almost normal appearances. The changes in the hair follicles in this case are shown in Plate XXVIII, fig. 8, and Plate XXX, fig. 17. $\times 100$.
- „ 13. Section of skin showing the filter-like depression at the mouth of the hair follicle, and the sebaceous glands. Note the absence of hyper-keratosis of the lining epithelium of the funnel of the follicle. The skin clipping was taken from a labourer with an apparently normal skin. $\times 50$.
- „ 14. Section of a hair follicle from a case of phrynoderma showing the mononuclear lymphoid cells in the perifollicular region, mostly in the lower part. $\times 50$.

EXPLANATION OF PLATE XXX.

Figs. 15 to 18. Sections of papules showing hyper-keratosis of the hair follicle dilatation of their funnels by the keratotic plugs and cellular infiltration in the perifollicular tissues. Remnants of the sebaceous glands can be seen in Fig. 17. Note the coiled and atrophic hair, cross sections of which can be seen in the substance of the follicular plug in Fig. 16, and the atrophic lower part of the follicle, separated from the upper part by delicate fibrous tissue, in Fig. 18. $\times 50$.



Fig. 16.



Fig. 16.



Fig. 16.



Fig. 17

(iv) *Sweat glands*.—Changes in the sweat glands were not a marked feature (see Plate XXIX, fig. 12). In some instances, however, the coil glands were few in number, their epithelium was flattened or shrunken and irregular, and the lumen dilated; the cells were not granular in appearance and the general impression obtained after studying the serial sections was that the glands were not actively secreting. Slight round cell infiltration around the sweat glands was present in one instance. The funnel-like depressions in the epidermis containing the sweat pores was filled to a varying extent with the hyper-keratinous material. The spiral cleft between the prickle and horn cells, representing the upper part of the sweat duct, was sometimes found occluded by cornified material or was surrounded by cells containing keratohyaline granules. In one case, the upper part of some of the ducts in the corium was surrounded to a varying extent by bands of epithelium from the epidermis.

(v) *Hair follicles and hair*.—Marked changes were found in the hair follicles in all instances; the number of follicles affected and the degree of changes present, however, varied. The mouths of the hair follicles showed marked hyper-keratinization of the lining epithelium; as a result, the funnels of the follicles were widened and were plugged by dense masses of horny tissue (see Plate XXX, figs. 15 to 18). Closer examination revealed that the follicular plugs consisted of concentric lamellæ of flattened cornified cells in which there were no nuclei; in one case, however, the outer layers of the plug, which generally were not dense, showed few persistent nuclei. Instead of the filter-like depression (see Plate XXIX, fig. 13), slight or marked elevations were often found at the mouths of the affected follicles. Sections of the coiled and atrophic hairs (see Plate XXX, fig. 16) or broken hairs were seen in the substance of some of the follicular plugs, but pustulation in the latter was not noted in any instance.

Serial sections showed that the lower part of the affected follicles was atrophic and was often separated from the upper part of the follicles by delicate fibrous tissue (see Plate XXX, fig. 18). In some places, however, the lower third of the follicles showed moderate hypertrophy of the external root sheath. Pieces of atrophied hairs were seen in the lower part of some of the follicles.

Varying degrees of cellular infiltration—mostly fibroblasts and mononuclear lymphoid cells—were often present in the loose perifollicular tissue; poly-morpho-nuclear leucocytes, eosinophils, and evidences of hæmorrhage were not noticed in any instance (see Plate XXIX, fig. 14, and Plate XXX, figs. 15 to 18).

Briefly described, the main histo-pathological features of this condition consisted of a superficial hyper-keratosis of the epidermis and hair follicles, marked dilatation of the funnels of the pilo-sebaceous follicles by keratotic plugs, atrophy of the sebaceous glands, and impaired function of the sweat glands.

COMMENT.

It may be seen from the above description that the clinical and the anatomical features of phrynoderma, as met with here, are similar to those described by Frazier and Hu, Loewenthal, Nicholls, and others. The follicular lesions were reported to be frequent in young adults by Frazier and Hu (1934, 1936); in the present series of cases, however, the condition appeared to be equally common in

children of both sexes, the youngest being a child of 2½ years. Loewenthal (1935a), Nicholls (1934) and Wright (*loc. cit.*) found this condition common in children.

The papules, which constitute the important clinical manifestation of the condition, arise from pilo-sebaceous follicles as a result of a non-inflammatory hyper-keratosis of the lining epithelium. The mouths of the follicles gradually become dilated and plugged by the dense masses of horny tissue and the resulting mechanical pressure appears to be responsible for the atrophy of the lower part of the follicles. The perifollicular infiltration is probably secondary to the irritation of the keratotic plug in the orifice of the hair follicle. From the histological examination, atrophy of the sebaceous glands appears secondary, the primary pathological feature being hyper-keratinization of the epidermis and hair follicles. As pointed out before, sebaceous glands could still be seen in some follicles, the mouths of which showed varying degrees of hyper-keratinization (see Plate XXVIII, figs. 7 to 10). The absence of sebaceous cysts in connection with the damaged follicles in all instances would suggest that, apart from the mechanical factor, the nutritional deficiency is also primarily responsible for the atrophy of the sebaceous glands. Bommer (1934) attributed atrophy of sebaceous glands in the skin to a deficiency of vitamin A in the diet and Nicholls (1934) thought that the sebaceous glands require a fat-soluble vitamin for their secretion. The absence of marked pathological changes in the sweat glands suggests that the absence of sweating noted in the areas of the follicular lesions is mostly due to functional disturbances in the coil glands.

Similar follicular keratotic lesions have been described by Nicolau (1918), Wiltshire (1919), Aschoff and Koch (1919), Theodorescou (1928), Scheer and Keil (1934), and other workers in cases of scurvy. In reviewing the literature on the subject, Scheer and Keil (*loc. cit.*) pointed out that in scurvy the vascular lesions, which are the characteristic feature of the disease, are mainly present in the capillaries of the perifollicular region, and the hyper-keratosis which results is mainly limited to the hair follicles; on the other hand, the earlier follicular lesions of scurvy may not be distinguishable clinically from those observed in *phrynoderma*. A number of other conditions, such as *Ichthyosis follicularis*, *Lichen plano-pilaris*, *Pityriasis rubra pilaris*, *Keratosis supra-follicularis*, *Lichen spinulosus*, etc., characterized by the presence of horny plugs at the orifices of the hair follicles forming small papules, have been described (Macleod, 1920). It is, therefore, desirable to establish, beyond doubt, the specific nature of *phrynoderma*.

As previously noted, most observers have described *phrynoderma* as occurring in association with xerophthalmia and keratomalacia. The beneficial results of the therapeutic administration of cod-liver oil on the papular eruption, reported by Loewenthal (1933a and b) and Frazier and Hu (1931, 1934, and 1936), strongly suggest that the cutaneous manifestations are part of the syndrome of avitaminosis-A, and are not merely coincident symptoms. The primary non-inflammatory hyper-keratosis of the epithelium of the epidermis and hair follicles in *phrynoderma*, and the involvement of the cutaneous glands and hair derived from the ectoderm, are similar to the morphologic appearances seen in vitamin-A deficiency. On the other hand, investigations carried out by workers in these Laboratories (Aykroyd and Rajagopal, *loc. cit.*; Aykroyd and Krishnan, 1937) show that it is difficult to explain the incidence of *phrynoderma* on the hypothesis that vitamin-A deficiency is the sole cause. Thus, the condition is very rare in school children in Calicut on

the west coast of Madras, although xerophthalmia is rather more common than in other districts. Investigations in children's hostels revealed no definite association between the frequency of phrynoderma and xerophthalmia, and between the former and vitamin-A and carotene intake. Loewenthal (1933b) pointed out that in persons on an almost fat-free diet which is rich in vitamin-A content, the papulo-follicular dermatosis was not seen.

Experimental evidence which would throw light on the relation between cutaneous lesions as described and diet deficiency is lacking. Smith (1932) and Smith and Sprunt (1935) noted atrophy of the sebaceous glands in the tails of vitamin G (B₂)-deficient rats. Similar but less marked changes were also noted in vitamin-A and vitamin-B₁ deficiency (Smith and Sprunt, *loc. cit.*). Further experimental work would probably throw some light on the subject.

The absence of vascular changes or hæmorrhages in the perifollicular tissues, even in the advanced stages of the papular eruption in phrynoderma, rules out the anti-scorbutic factor from the ætiology of the condition. The clinical features of *Ichthyosis follicularis*, *Lichen plano-pilaris*, *Pityriasis rubra pilaris*, and *Acne vulgaris* are different from those of phrynoderma, so that difficulty rarely arises in the differential diagnosis of the latter. In the light of present knowledge, it would be interesting to study the ætiology of some of the conditions, such as *Lichen pilaris* (Smith, E. C., 1932), *Keratosis supra-follicularis*, *Lichen spinulosus*, etc., described as separate entities.

It is generally agreed that phrynoderma is a separate entity associated with malnutritional states. But, in view of divergent opinions regarding its ætiology, further study is required to determine the exact nature of the condition.

SUMMARY AND CONCLUSION.

The clinical and histo-pathological features of a papulo-follicular dermatosis described as '*phrynoderma*', associated with malnutritional states, were studied. The condition was found both in children and adults, mostly on the extensor surfaces of the arms, thighs and upper part (postero-lateral aspect) of the forearms near the elbows. An acne-like eruption on the face was not a conspicuous feature.

Histologically, the condition was characterized by a superficial non-inflammatory hyper-keratosis of the epithelium of the epidermis and hair follicles, distension of the mouths of the pilo-sebaceous follicles by horny plugs, atrophy of the sebaceous glands and impaired function of the sweat glands.

The condition is probably a manifestation of a nutritional deficiency in which lack of vitamin A is an important factor.

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THE SPECTROPHOTOMETRIC METHOD OF ASSAYING VITAMIN A AND CAROTENE WITH FURTHER DATA ON THE VITAMIN-A ACTIVITY OF INDIAN FOODSTUFFS.

BY

N. K. DE.

(*Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.*)

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In a previous communication (De, 1935), a simple spectrophotometric method of assaying vitamin A and its precursor carotene was described. Subsequently the method was, with slight modifications, applied in the assay of the carotene content of over 100 samples of vegetable foodstuffs (De, 1936*a* and *b*). In the present paper, further data regarding this method of assay are presented, and the results of the investigation of the vitamin-A potency of a further 70 foodstuffs are included. During the routine examination of foodstuffs, additional evidence has appeared in support of the validity of the technique and a few more points of technical importance have been brought to light.

Of the various methods of assaying vitamin A and carotene, viz., biological, chemical, and spectrographic, the last named has in recent years been accepted as the most satisfactory (Morton, 1935 ; and other workers). In vitamin-A assay work by non-biological laboratory methods, it is necessary that vitamin A and carotene should be estimated separately. There is no satisfactory chemical method for distinguishing and estimating them separately when they occur simultaneously. Double estimations are, however, needed only in the analysis of animal products in which vitamin A and carotene occur together. In the case of vegetables, vitamin A as such does not occur (Wolff *et al.*, 1930 ; Moore, 1931 ; De, 1935 ; and other workers) ; hence carotene alone has to be estimated.

Although vitamin A and carotene resemble each other in their physiological action, they are quite different in their chemical and physical properties. As regards molecular constitution, vitamin A is an unsaturated alcohol (colourless), while carotene is a yellow unsaturated hydrocarbon pigment. The former shows a characteristic absorption spectrum with a single maximum at 3,280 A. U. The latter (like the xanthophylls and many other lipochrome pigments) besides its specific absorption in the visible region shows some weak bands in the long and

short ultra-violet. Estimation of carotene from studies of absorption in the visible region is not influenced by the presence of vitamin A, while an accurate assay of vitamin A contained in animal products, particularly richly pigmented ones (butter, egg-yolk, blood, etc.), is rather a complicated problem, as the carotenoids occurring simultaneously may markedly influence the gross absorption at 3,280 A. U. To avoid this difficulty two courses can be followed: (1) to deduct from the gross absorption at 3,280 A. U. that contributed by the carotenoid pigments, or (2) to devise a simple chemical process that will isolate vitamin A from the interfering pigments.

DEDUCTION OF ABSORPTION DUE TO THE CAROTENOIDS.

Gillam (1934) has used this principle in estimating vitamin A contained in various samples of butter. The gross intensity of absorption at 3,280 A. U. was measured; this was in part due to vitamin A and in part due to carotene accompanied by traces of xanthophylls. The absorption at 3,280 A. U. due to the latter was obtained by dividing the $E \frac{1 \text{ per cent}}{1 \text{ cm.}}$ value at 4,500 A. U. to 4,600 A. U. by 6.5. In the present experiments absorption spectra of several solutions of pure carotene (International Standard Preparation, 1931) were studied and a similar ratio (1/6 to 1/7) between the intensities of absorption at the region of 3,300 A. U. and 4,500 A. U. to 4,600 A. U. was recorded. This procedure of estimating the non-vitamin absorption will serve well, provided the interfering pigments consist mainly of carotene as in the case of butter. In the analysis of other test: ... (etc.) where carotene is associated with high proportions of xanthophylls and similar pigments, it would hardly be justifiable to evaluate the irrelevant absorption in the same way. We have little knowledge regarding the true value of the correction factor that would be applicable in such complicated cases; and any attempt to remove these non-hydrocarbon pigments by the Kraus separation will simultaneously remove part of the vitamin A present.

PARTITION BETWEEN VITAMIN A AND CAROTENE BY THE PHASE TEST.

It has been reported by several workers (Wolff *et al.*, *loc. cit.*; Rosenthal *et al.*, 1935; and other workers) that vitamin A and carotene can be separated from a petroleum ether solution by repeated phase tests between alcohol and petrol, vitamin A going to the former solvent while carotene is left in the latter. Experiments were undertaken to study the solubility of vitamin A in alcohol (90 per cent) and petroleum ether (B. P. 60°C. to 80°C.). It was found that vitamin A is highly soluble, and also more or less equally soluble in both these solvents. As such, strictly speaking, the above procedure of depriving petroleum ether of vitamin A by alcohol is not a real Kraus separation. Vitamin A distributes itself more or less uniformly in both the phases and in each operation, with an equal volume of 90 per cent alcohol, roughly half the amount of vitamin A would be removed. It can thus be anticipated that for a quantitative separation (within 1 per cent error), using each time an equal volume of alcohol, at least seven operations would be needed, and the percentage concentration of vitamin A in petrol would fall roughly in the following geometrical progression: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and so on. This

has been experimentally confirmed as follows: petroleum ether solutions of vitamin A (unsaponifiable fractions of halibut-liver oil) were washed repeatedly with an equal volume of 90 per cent alcohol (ethyl and methyl) until no vitamin A was left behind in the petrol as tested colorimetrically. For a quantitative partition as many as 7 to 10 washings were required. Partition in the reverse direction—alcohol to petrol—was also investigated and found to be equally effective. Recently, Gillam and Senior (1936) have investigated this problem thoroughly, and report similar results which were published while the manuscript of this paper was being prepared. The partition method was then tried on several petroleum ether solutions of Haliverol dissolved directly. Even after 10 washings with 90 per cent alcohol, only a part of vitamin A was removed from the petrol. This discrepancy is probably due to the fact that petrol, as compared with alcohol, is a much better solvent of fats and as such exerts a stronger pull on the free fat, while the fat-soluble vitamin A preferentially adheres to the parent substance. Similarly, Gillam and Senior (*loc. cit.*) report that the presence of large quantities of sterols (cholesterol) affects the partition co-efficient of vitamin A, petrol retaining the greater portion of vitamin A. The solubility of a commercial sample of cholesterol was investigated and found to be greater in petrol than in alcohol. It can be concluded from these observations that the presence of any foreign material having a greater solubility in petrol will affect the partition in the same way.

The question now arises, how far this partition method may be useful in routine vitamin-A and carotene assay work. The possibility of its use is certainly very much limited, like that of the previous method, since xanthophylls and other pigments will invariably accompany vitamin A to the alcohol phase.

REMOVAL OF THE PIGMENTS BY CHARCOAL ADSORPTION.

It has long been known that charcoal is a very powerful adsorbent for various pigments. Experiments were conducted to discover if charcoal adsorption could effect a quantitative separation of vitamin A from the associated pigments. Applications of this method, though not with strict quantitative purposes, have already been mentioned by several workers (Gillam *et al.*, 1933; and others). It was observed that treatment with charcoal destroys both vitamin A and carotenoids, the latter, however, being much more quickly destroyed than the former. Stability to charcoal could be much increased by addition of hydroquinone. Several ether and petroleum ether solutions of vitamin A (cod-liver oil) and carotene (red-palm oil), the proportionate concentrations of which ranged from 1:1 to 1:5, were treated with a minimum amount of charcoal, slightly shaken, filtered, and the precipitate washed 3 to 5 times, the whole operation being carried out as quickly as possible. In each of these attempts, carotenoids were removed completely, leaving behind a clear colourless solution of vitamin A. There occurred no appreciable loss of vitamin A, as evidenced by the arsenic-trichloride test even when the test solutions contained as low as 2γ to 5γ of vitamin A per c.c. A petroleum ether solution, containing per c.c. 4γ and 20γ of vitamin A and carotene respectively, was decolorized by charcoal (0.2 g. charcoal per c.c.: shaken for 2 minutes, filtering and washing for 8 minutes) and examined spectrographically. The loss of vitamin A in this case was only 9 per cent. This method of separation of vitamin A from all classes of associated pigments appears to be fairly satisfactory.

and with sufficient precautions it may be reliably applied in routine work. Further data about the charcoal adsorption of vitamin A and carotene will be reported later.

STABILITY OF VITAMIN A AND CAROTENE TOWARDS LIGHT.

It is a well-known fact that both vitamin A and carotene are subject to destruction by light. It is thus of great technical importance to adjust the conditions so that during the photometric study concentrations of vitamin A or carotene are least affected by exposure to light. Precise knowledge of how this can be done is still lacking, although spectrophotometric methods are nowadays being extensively used. Accordingly, the following investigation was undertaken: roughly equal quantities (about 15.0 γ) of vitamin A and carotene (in cod-liver oil and red-palm oil respectively) dissolved in equal volumes (10 c.c.) of different solvents, viz., chloroform, ether, petroleum ether, and alcohol, were exposed to the total radiations of a quartz mercury vapour lamp (Heraeus: current 3.5 amperes at 220 D. C.) under identical conditions (distance 20 cm., thickness of the layer of solution about 1.5 cm.) and their stabilities to light compared. The evidences for the destruction of vitamin A and carotene were respectively a negative arsenic-trichloride reaction and the disappearance of all yellow colour. The results of several experiments are set forth in Table I:—

TABLE I.

Effect of ultra-violet irradiation on vitamin A and carotene.

Solvent.	APPROXIMATE MINIMUM TIME (IN MINUTES) REQUIRED FOR COMPLETE DESTRUCTION: CONCENTRATION, ROUGHLY 15 γ PER C.C.	
	Vitamin A.	Carotene.
Chloroform (Merck)	7 to 9	1.5 to 3
Ether (Kahlbaum) ..	23 to 25	35 to 40
Petroleum ether (B. P. 60°C. to 80°C.).	22 to 26	20 to 25
Alcohol (96 per cent ethyl).	20 to 25	22 to 27

From Table I, it is seen that both vitamin A and carotene are easily destroyed by ultra-violet irradiation in all the solvents, and that the effect is very much more rapid in chloroform. The stabilities of vitamin A and carotene are more or

less alike in alcohol and petrol. In chloroform, the photo-chemical destruction of carotene is much quicker than that of vitamin A, while in ether, carotene is slightly more stable than vitamin A (De, 1935). Inactivation by ultra-violet irradiation of vitamin A and carotene in the presence of hydroquinone was also investigated and found to be considerably retarded. Irradiation of carotene by visible light alone was found to be very much less effective, while in the case of vitamin A there occurred practically no destruction (De, 1935).

Solutions containing varying amounts of vitamin A and of carotene in the absorption cells (1 cm. quartz vessels used with the Hilger's sector photometer) were then exposed to the total radiations of the source (spark between Tungsten steel electrodes, gap 4 mm.) under identical conditions as in a photometric study. In all cases an exposure of 10 minutes was given. In chloroform roughly 40 to 95 per cent of the potencies were destroyed, as judged by colour test, even when the concentrations were as high as 5γ to 10γ per c.c. Chloroform is thus quite unsuitable as a solvent for spectrographic analysis. Even within a few hours, under ordinary conditions, vitamin A and carotene deteriorate appreciably in chloroform. In other solvents, viz., ether, petroleum ether, and alcohol, the loss was not noticeable using concentrations of 5γ to 7γ per c.c., while with dilute solutions (1γ to 2γ per c.c.) the destruction was definitely marked. Exposures to the source, after filtering off the short ultra-violet radiations by interposing a thick transparent glass plate, were found to be absolutely harmless in the same conditions. While assaying carotene by studies of absorption at 4,500 A. U. to 4,600 A. U., it would be preferable to use only the visible radiations.

In order to safeguard the accuracy of the technique, while using the total radiations, it is necessary that the test solutions should neither be too diluted, nor too concentrated, since in both cases appreciable destruction may occur; in the former even a short exposure is enough to affect the concentration, while in the latter longer exposures will be necessary and this may cause much destruction. It appears, however, that previous treatment with pure hydroquinone may solve this difficulty. The addition of a foreign material must not, however, interfere with the study of absorption. To elucidate this question, petroleum ether solutions of hydroquinone, irradiated and non-irradiated, were spectrographed and found to be transparent in the regions concerned.

It may be mentioned that when the concentrations of the test solutions can be previously guessed from the colour or the nature of the material, it would be safer and economical to give only a few exposures, adjusting the variable sector about the position computed, instead of photographing for all its readings. It appears also that concentrations of the order of 5γ to 7γ of the potent factor per c.c. (giving an extinction co-efficient equal to about 1) are most suitable for spectrography.

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THE ASSAY OF FOODSTUFFS.

Technique.

The principle and the technique were in general the same as those described previously (De, 1935, 1936a). Test solutions of vitamin A were obtained in ether

or pétroleum éther, those of carotene in the last-named solvent. The amount of vitamin A or carotene present was estimated by determining the difference between the extinction co-efficients at 3,280 A. U. and 4,550 A. U. to 4,600 A. U., respectively, before and after irradiation. The absorption cell was filled with the test solution treated with a little spectrographically pure hydroquinone, while the compensating cell was filled with the extract from which vitamin A or carotene had been completely removed by irradiation for periods just sufficient, under a mercury vapour lamp. For calculations, the following formulæ were used: $E \frac{1 \text{ per cent}}{1 \text{ cm.}} = 1,600$ for pure vitamin A and 2,500 for pure carotene (Carr and Jewell, 1933; Gillam, 1935). In the photometric study of carotene solutions, the short ultra-violet radiations of the source were filtered off by interposing a thick transparent glass plate.

Extraction.

The vitamin-A assay was carried out on materials of animal origin only, since vegetable foods have been found to be devoid of vitamin A (Wolff *et al.*, *loc. cit.*; Moore, *loc. cit.*; De, 1935; and other workers). Test solutions from potent oils and fats were prepared directly by dissolving them in a suitable solvent. Other materials were saponified on a water-bath under reflux, with slightly excess of colourless 20 per cent alcoholic potash, and the unsaponifiable fraction extracted with light pétroleum ether. After washing several times with distilled water, the extract, freed from moisture, was concentrated to a suitable volume by low heat in an atmosphere of carbon dioxide gas. The final solution, if appreciably coloured, was divided into two parts. Part 1 was decolorized by shaking with a minimum amount of charcoal and used for vitamin-A assay. Part 2 was freed from the non-hydrocarbon pigments by the usual procedure (De, 1935, 1936a) and used for carotene estimation. Direct extraction of vitamin A and carotene by alcohol and petrol was tried on a few animal products. This procedure was not at all encouraging, since the extracts, after concentration, were insufficiently clear and transparent, owing to the presence of too much of fatty substances. This difficulty, due to excess of fats, was not experienced in the case of vegetable foods (except oils very poor in carotene), and carotene was extracted directly with absolute alcohol and then repeatedly with pétroleum ether. The extracts were collected together in a separating funnel, and a few c.c. of water were added, bringing the alcohol to about 85 per cent. On shaking, carotene went to the petrol phase and xanthophylls to alcohol. The carotene solution was then purified from the irrelevant pigments by repeated phase tests, and by adsorption on calcium carbonate.

Several workers have remarked that saponification is necessary to free carotene from the combined xanthophylls and their esters. No appreciable difference was noted in the study of a few test materials (amaranth, red-palm oil, and egg-yolk), with and without saponification. It is possible that these interfering substances do not occur in any significant amount, or probably they are removed by adsorption on calcium carbonate.

The present vitamin-A assay work forms a part and is a continuation of the food survey work in progress in these Laboratories. In this paper data about the

vitamin-A activities of some 70 additional foodstuffs are reported. The results are set forth in Tables II and III :—

TABLE II.

The vitamin-A and carotene content of some animal products.

Material.	Vitamin-A content per gramme (γ).	Carotene content per gramme (γ).
Adexoline capsules	3,125.00 (per capsule).	..
Cod-liver oil (Norwegian)	562.50	..
„ (different sample)	400.00	..
„ („ „)	314.00	..
„ („ „)	312.00	..
„ („ „)	187.00	..
„ („ „)	156.00	..
Giant-ray-liver oil (<i>Decerobatus</i> sp.)	Trace only	..
Haliverol (Parke, Davis & Co.)	12,702.00	..
Saw-fish-liver oil (<i>Pristis</i>)	170.00	..
Skate-liver oil	437.00	..
Chamban (whole fish)	Trace only	..
Dolphin oil (from head and brain)	Trace only	..
Herring oil (<i>Clupea toli</i>) (whole fish)	1.00	..
Mackerel oil (<i>Rastrilliger-kanangurta</i>) (whole fish)	Trace only	..
Sardine fish oil, 3 years old (whole fish)	2.50	..
Beef liver	131.00	..
„ muscle	0.23	Trace only
Sheep liver	87.00	..
„ muscle (mutton)	0.12	Trace only
Butter (from local dairy)	14.60	0.80
Ghee („ „ „)	10.40	Nil.
Skim-milk powder (New Zealand)	Nil	Nil.
Hen's egg (English poultry) (yolk only)	15.00	32.60
„ „ (country „) („ „)	12.70	28.40

TABLE II—*concl'd.*

Material.	Vitamin-A content per gramme (γ).	Carotene content per gramme (γ).
Crab (caught from the local hill streams) ..	Trace only	13.00
Chasker fish* (flesh only) (from West Coast) ..	Trace only	0.14
Aila fish* (<i>Scomber microlepidotus</i>) (flesh only) (from West Coast).	0.25	0.10
Keelakkan fish* (<i>Sillago sihama</i>) (flesh only) (from West Coast).	Trace only	0.10
Keluthi fish* (cat fish) (flesh only) (from West Coast)	Trace only	Trace only
Madava* (Mugil) fish („) („ „ „)	0.10	0.20
Prawn „ („) („ „ „)	Trace only	Trace only
Sorra fish* (<i>Carcharias</i>) (flesh only) (from West Coast)	0.20	0.10
Panna „ * (<i>Ottolithus</i> sp.) („) („ „ „)	Trace only	Trace only

* These are Tamil or Malayalee words. For chasker the scientific name could not be traced.

That carotene may exist in different isomeric states (α , β , and γ forms, the second being about twice as potent as either the first or third) was not considered; only the total carotene content was estimated. One γ (0.001 mg.) of these carotene figures will correspond to about 1 International Unit, which is equal to 0.6 γ of pure β carotene. One γ of the vitamin-A data = 2.6 I. U. (De, 1937).

TABLE III.

The carotene content of some fruits and vegetable foods.

Material.	Carotene content per gramme (γ).
FRUITS.—	
Dates (Persian, preserved)	6.0
Guava (hill variety), ripe	Trace only
„ (country) „	Trace only
Jack-fruit (ripe)	5.4
Mango, Ankola (from Bombay)	18.0
„ „ (after further ripening for 1 week due to keeping in cold storage).	24.4

TABLE III—*contd.*

Material.	Carotene content per gramme (γ).
FRUITS—<i>concl'd.</i>	
Mango, Ankola (after further ripening for 2 weeks due to keeping in cold storage).	32.0
„ „ Alphonso (from Bombay) ..	25.0
„ „ (after further ripening for 1 week due to keeping in cold storage).	32.0
„ South Indian (Nilam), ripe ..	61.6
„ „ „ (Kotaiyam), ripe ..	54.0
„ „ „ (Mysore Gundoo), ripe ..	51.2
„ „ „ (Salem graft) „ ..	48.0
„ „ „ (Malgoa) „ ..	28.0
„ „ „ (Kotagiri) „ ..	23.3
„ „ „ (Salem village) „ ..	22.5
„ „ „ (Malabar) „ ..	16.0
„ „ „ („), partly ripe ..	3.9
Plums (yellow variety)	2.3
„ (white skin)	Trace only
Pineapple (tender)	<i>Nil</i> or trace
„ (ripe)	0.6
VEGETABLES: MISCELLANEOUS.—	
Red-palm oil (Burma)	569.0
„ „ (Malaya)	520.0
Drumstick leaves (<i>Moringa oleifera</i>) ..	113.3
Fenugreek leaves (<i>Trigonella fœnum-græcum</i>) ..	38.6
Ipomœa (<i>Ipomœa reptans</i>)	32.7
Mint (<i>Mentha viridis</i>)	27.0
Sandakai (<i>Solanum torvum</i>), dry	7.5
Brinjal, thorny (<i>Solanum xanthocarpum</i>) ..	4.8
Ginger, new fresh	4.0

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Prawn „ („) („ „ „)	Trace only	Trace only
Sorra fish* (<i>Carcharias</i>) (flesh only) (from West Coast)	0.20	0.10
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in extracts from biological materials. Baumann and Steenbock (1933), MacWalter (1934), Nøtveit (loc. cit.) and other workers have studied the action of oxidation, heat, and irradiation on several vitamin-A concentrates. They report that in effecting selective destruction of vitamin A, ultra-violet irradiation gave satisfactory results, while the other two agents caused marked increase of absorption in the shorter ultra-violet and the products also showed absorption at 3,280 Å. U. In the course of the present study, extracts (concentrated) of the unsaponifiable fractions of several vitamin-A-free oils (gingelly oil, sardine fish oil, Chamban fish oil, etc.) were directly spectrographed. They all showed appreciable degrees of absorption in the region 3,700 Å. U. to 3,000 Å. U. The absorption increased very rapidly towards the shorter ultra-violet, while in the visible region it was absent or negligible. On irradiation, a slightly increased absorption was often noted in the shorter regions with little or no effect (increase or decrease of absorption) above 3,000 Å. U.

These observations show that irradiation for a reasonable period does not materially influence the irrelevant absorptions at 3,280 Å. U. and 4,500 Å. U. to 4,600 Å. U. The effect of irradiation on these vitamin-A-free oils direct was, however, found to be uncertain, since it sometimes caused an increased absorption throughout the whole of the ultra-violet. In the case of very potent oils (cod-liver oil, Haliverol, etc.) no such difficulty was experienced.

The gross intensity of absorption at 3,280 Å. U. and at 4,500 Å. U. to 4,600 Å. U. is in part due to vitamin A and carotene respectively and in part due to foreign materials which exhibit absorption, not necessarily selective in the regions concerned. In order to estimate the correct value of absorption due to vitamin A or carotene, it is necessary to remove either the foreign materials or the factor under test. Complete removal of impurities by chemical means and by subjection to very low temperature, etc., is an extremely difficult task and still remains uncertain. Strain (1935) reports that even carefully prepared solutions of carotene may contain various colourless impurities. In the present state of knowledge, ultra-violet irradiation, on preferably the unsaponifiable fractions of the poor and moderately rich materials, appears to be a very convenient and satisfactory method of removing vitamin A and carotene selectively. Determination of the difference between the absorptions before and after irradiation will thus give a correct measure of the potent factor present. The method has been applied in the analysis of very potent materials, as well as very poor ones, potency ranging from 13,000 γ to 0.1 γ per gramme. The results are quite satisfactory and encouraging.

From Tables II and III, it is seen that, with the exception of liver, liver oils, butter, ghee, and egg-yolk, flesh foods in general are very poor in vitamin A. Green leafy vegetables, ripe fruits such as mangoes, oranges, tomatoes, papaya, etc. (De, 1936a), are particularly rich in carotene; their carotene content in most cases approaches or exceeds the vitamin-A activity (carotene and vitamin A *strictu sensu*) of egg-yolk or milk fats. Red-palm oil stands pre-eminent in the list of vegetable foodstuffs and its potency is comparable to that of an average sample of medicinal cod-liver oil. From the results of the assays of ripe and green fruits, it appears that vitamin-A potency (carotene content) increases during ripening. The carotene content of the fruits analysed was found to be proportional to the degree of ripeness and the intensity of the yellow coloration.

Biological experiments by Morgan and Smith (1928), House *et al.* (1929), and other workers on tomatoes, ripened both artificially and naturally, resulted in similar conclusions.

Various samples of medicinal cod-liver oil (Norwegian) analysed showed wide differences in potency, vitamin-A content ranging from 150 γ to 600 γ per gramme. This is quite possible, since the vitamin-A content of such oils may vary according to the age, diet, and source of the fish caught; and a deterioration may also occur which is due to mere storage for prolonged periods. It is strange to note that the sample of giant-ray-liver oil was devoid of vitamin A. The lay public goes simply by the name and never questions the potency of an oil they are advised to take. It would be of public health value, if manufacturers were obliged to state what are the original potencies of their products and to what extent these are maintained under the conditions of filling and storage.

SUMMARY.

1. Experiments were made to study the partition of vitamin A from petrol to alcohol. For a quantitative partition saponification was found to be necessary, and as many as 7 to 10 washings were required. The method applied to unsaponifiable fractions was equally effective in the reverse direction, namely, from alcohol to petrol. It appears that the presence of foreign materials having unequal solubilities in the two solvents affects the partition co-efficient of vitamin A between the two phases.

2. Vitamin-A solutions could be freed from various interfering pigments by adsorption on charcoal without causing appreciable loss. This procedure, with due precautions, was found to be fairly satisfactory for use in routine work.

3. Solutions of vitamin A and carotene in different solvents were irradiated and their stabilities to light compared. Experiments were made to find out the conditions, viz., solvent, concentration, the time of exposure, etc., that are most suitable for spectrophotometric studies. Both vitamin A and carotene are highly unstable in chloroform. Convincing reasons are given why use of chloroform should be avoided in spectrographic work.

4. Considerable evidence was obtained in support of the validity of the technique. It is concluded that the difference in absorption before and after irradiation gives a very accurate measure of the vitamin A or carotene present. This principle applied, on preferably the unsaponifiable fractions of poor and moderately rich materials, eliminates any absorption due to impurities.

5. The vitamin-A and carotene contents of some 70 additional foodstuffs have been assayed using the modified and improved technique as described.

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THE ASSIMILATION OF VITAMIN A AND CAROTENE BY
RATS FROM SOME COMMON FOODS WITH A NOTE ON
THE CONVERSION FACTOR I. U./E., PROPOSED
BY THE INTERNATIONAL VITAMIN
CONFERENCE.

BY

N. K. DE.

(*Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.*)

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IN previous communications (De, 1936, 1937) it has been pointed out that the vitamin-A potency (as indicated by the carotene content) of many vegetable foodstuffs, particularly green leafy vegetables, and ripe fruits such as mango, papaya, orange, tomato, etc., may approach or even exceed that of egg-yolk and milk fat. A few samples of red-palm oil tested have been found to be very rich in carotene, and their carotene values indicate that these oils are as potent as an average sample of medicinal cod-liver oil. Hence carotene-rich vegetable foods, which in general are much cheaper and more easily available than animal products, appear to be of great importance as a source of vitamin-A activity. Although it might be expected that vitamin A from animal matter would be better utilized than its precursor in vegetable foods, we have so far no precise knowledge on this question.

Vegetable foods, because of their cellular structure, are in general less digestible than animal foods. The faeces, particularly those of young children, are frequently found to contain undigested materials derived from green vegetables and fruits. It seems probable that both vitamins and minerals must be lost through incomplete digestion, and hence the nutrients of vegetable foods would be less efficiently utilized. That vitamin A and carotene may be lost through incomplete digestion has been reported by several workers (Rowntree, 1930; Moore, 1931; Baumann and Steenbock, 1934; and others). The loss invariably occurred by excretion in the faeces; the urine was observed to be always devoid of vitamin A and carotene. The greatest loss as well as the greatest retention occurred with the greatest intake. Moore (1931) detected the presence of unchanged carotene throughout the alimentary tract (of rats), and the pigmented fat derived from the faeces was found to be biologically active, at a level corresponding to its apparent carotene content. Baumann and Steenbock (1934) report that only about 5 to 10 per cent of vitamin A ingested by vitamin-A-depleted rats

in a single dose of 170 to 1,700 International Units (I. U.) could be recovered in the liver; no storage in the liver resulted on feeding 34 I. U. Faecal excretion of vitamin A could account for only a small fraction, while the major portion was destroyed in the digestive tract. They report also that there is a parallelism between the absorption of vitamin A occurring in rats and that occurring in children as observed by Clausen (1933). Rowntree (*loc. cit.*) has found that vitamin A is not excreted in the urine of infants and young children, even when generous amounts are fed; without exception, faecal loss was noted varying from 2 to 12 per cent of the intake, and being apparently proportional to the loss of fat.

It has been observed that the biological activity of carotene depends on the nature of the solvent. The use of mineral oils as solvent considerably hinders the absorption of carotene, as shown by the recovery of carotene from the faeces (Dutcher *et al.*, 1934). So also different biological values for pure carotene may be obtained by the use of different vegetable oils as solvent, or by different samples of the same solvent (Baumann and Steenbock, 1933; Culhane *et al.*, 1934; and other workers). There is no very definite evidence as to stability of carotene in these tests, and it is impossible to say whether the difference is due to variation in the utilization or variation in the stability of the carotene.

Ahmad (1931) reports that the absorption of carotene (in rats), fed in ethyl-laurate solution, was considerably affected by the absence of fat in the diet. When carotene was fed in red-palm oil, in conjunction with fat-poor and fat-free diets, about the same amount—40 per cent of the carotene ingested—was recovered from the faeces. Greaves and Schmidt (1935) report that the fat content of a diet, within the limits of 3 to 20 per cent, does not materially influence the absorption of carotene when the latter is fed in cotton-seed oil, at a level of 5 γ to 15 γ per rat per day.

From the literature, it appears that there has so far been very little work done in this field. Accordingly, an investigation was undertaken to study the relative utilization of vitamin A and carotene by rats from a number of natural foods. The present work throws some light on this point, which is of obvious importance. Simultaneously, experiments were carried out to study how the difference in body-weights of the experimental animals, and the fat, vitamin B and the mineral content of the diet may influence the absorption of vitamin A and carotene.

EXPERIMENTAL.

Several groups of stock rats were kept on a vitamin-A-free basal diet for 7 to 12 days, until the faeces were free from vitamin A and carotene. The animals were then given diets containing known amounts of vitamin A or carotene. The total amount of vitamin A or carotene lost by excretion in the faeces was determined. The difference between the amounts ingested and excreted was taken as a measure of the amount absorbed or retained in the body. Absorption or retention, in this sense, includes the amounts of vitamin A or carotene that are destroyed in the digestive tract. No loss was ever found to occur through the urine. The test materials were fed to groups of rats, for periods ranging from 3 to 5 days; in some cases absorption from a single feeding of the test substance was studied. The following materials were fed as sources of vitamin A or carotene: halibut-liver

oil, cod-liver oil, butter, egg-yolk, amaranth, papaya, orange juice, red-palm oil, and two mixed diets. The first, second, third, sixth, seventh, and eighth named materials were fed separately, before the main diets were supplied. Amaranth and egg-yolk were first mixed with small amounts of the vitamin-A-free synthetic diet, and fed to the animals before the bulk of the main diet was supplied. The carotene containing mixed diets was supplied in liberal quantities (known amounts), and the amount consumed calculated by weighing out the residue, loss of weight due to evaporation of moisture being accounted for.

The composition of the basal diet and the two mixed diets was as follows :—

<i>Basal diet.</i>	Parts.
Starch	63
Casein	20
Coco-nut oil	10
Salt mixture	5
Yeast	2
With tap water to drink	

<i>Mixed diet No. 1.</i>	Parts.
Cholam (<i>Sorghum vulgare</i>)	2·00
Red gram (<i>Cajanus indicus</i>)	0·25
Ground-nut (<i>Arachis hypogea</i>)	0·06
Amaranth (<i>Amaranthus gangeticus</i>)	0·01
Brinjal (<i>Solanum melongena</i>)	0·01
Bitter gourd (<i>Momordica charantia</i>)	0·01
Common salt	0·06
With tap water to drink	

<i>Mixed diet No. 2.</i>	Parts.
Raw polished rice	12·0
Cholam (<i>Sorghum vulgare</i>)	6·0
Red gram (<i>Cajanus indicus</i>)	1·0
Root vegetables	2·0
Leafy vegetables	8·0
Ground-nut (<i>Arachis hypogea</i>)	1·0
Soya bean (<i>Glycine hispida</i>)	1·0
Coco-nut oil	1·0
Butter-milk	10·0
Dried skim milk (New Zealand)	0·5
Jaggery	1·0
With tap water to drink	

This diet was devoid of vitamin A : no vitamin A was detected in the butter-milk used.

Synthetic diets, other than the basal diet, which were free from fat, yeast (vitamin-B complex), or salt mixture, were prepared by substituting starch for the factor eliminated from the basal diet. The mixed diet No. 1 was based on a diet given to children in famine camps in the Madras Presidency. Aykroyd and Krishnan (1936a) observed that a high percentage of the children consuming this

diet, supplying roughly 700 γ of carotene daily, showed symptoms of vitamin-A deficiency. It was felt to be of interest to determine carotene absorption from this diet. Mixed diet No. 2 is one of the 'cheap balanced diets' devised and investigated in this laboratory (Aykroyd and Krishnan, 1936b).

The results of the various experiments are shown in Tables I, II, and III, which supply details about the number of animals used, period of feeding, amount of vitamin A and carotene fed and the amount excreted in the faeces, etc. The investigation includes studies of (1) the absorption of vitamin A and carotene by rats from a number of natural foods and (2) the influence of several factors on the absorption of vitamin A and carotene. These were: body-weight of the experimental animals, level of vitamin-A or carotene intake, and the fat, yeast (vitamin-B complex), and mineral content of the diet.

The estimation of vitamin A and carotene in the various foods, as well as in the faeces, was carried out by the modified spectrophotometric method (De, 1935, 1937). Carotene from the various test materials was extracted directly, while vitamin A in general was extracted from the unsaponifiable fraction; in the case of halibut-liver oil and cod-liver oil, test solutions were directly prepared.

TABLE I.

1. *The absorption of vitamin A from butter (fat-rich diet).*

Days.	Quantity of vitamin A fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of vitamin A excreted in faeces (γ).	Total quantity of vitamin A fed (γ).	Total quantity of vitamin A excreted (γ).	Percentage of vitamin A excreted.	Percentage of vitamin A retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	105.6	2.5	542.4	17.8	3.3	96.7
2	105.6	3.8				
3	105.6	4.7				
4	112.8	3.7				
5	112.8	..				
6	0.0	3.1				
7	0.0	0.0				
8	0.0	0.0				

The group consisted of 6 rats of body-weights ranging from 130 g. to 170 g. They were fed on the basal diet with an addition of 2 g. of butter per rat per day. The diet including butter contained about 30 to 40 per cent of fat. The quantity of vitamin-A intake was about 18 γ per rat per day. The presence of a small

quantity of carotene in butter was disregarded. The fæces of the 5th and 6th days were tested together.

TABLE I—*concl'd.*2. *The absorption of vitamin A from cod-liver oil (fat-free diet).*

Days.	Quantity of vitamin A fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of vitamin A excreted in fæces (γ).	Total quantity of vitamin A fed (γ).	Total quantity of vitamin A excreted (γ).	Percentage of vitamin A excreted.	Percentage of vitamin A retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	82.2	..	411.0	19.2	4.7	95.3
2	82.2	..				
3	82.2	..				
4	82.2	14.2				
5	82.2	..	5.0	..	..	..
6	0.0	..				
7	0.0	5.0				
8	0.0	0.0				

The group consisted of 6 rats of body-weights ranging from 130 g. to 160 g. They were fed by hand with 1 drop of cod-liver oil per rat per day. The fat content of the basal diet was replaced by starch and sufficient quantities of the fat-free diet were supplied to the animals. The level of vitamin-A intake per rat per day was about 14.0 γ . The fæces of the first 4 days and of 5th, 6th, and 7th days were tested together.

TABLE II.

1. *The absorption of carotene from amaranth (large supply of carotene).*

Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of carotene excreted through the fæces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	800.0	137.0	1,752.0	1,004.0 + roughly 30 per cent of 1,004.0 = 1,305.0	74.0 (roughly)	26.0 (roughly)
2	576.0	372.0				
3	576.0	495.0				

The group consisted of 3 rats of body-weights ranging from 120 g. to 160 g. They were fed on the basal diet including 2 g. of fresh amaranth leaves per rat per day. The level of carotene intake per rat per day was about 195·0 γ . Examination of the faeces for carotene after the supply was stopped was not carried out. Experience showed that carotene excretion may continue for some days after the removal of a supply from the diet and 20 to 40 per cent of the total carotene excretion may occur during this time.

TABLE II—*contd.*2. *The absorption of carotene from amaranth (small supply of carotene).*

Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0·001 mg.).	Quantity of carotene excreted through the faeces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	72·0	15·0	381·0	207·0	54·3	45·7
2	77·0	60·0				
3	77·0	22·0				
4	88·0	36·0				
5	67·0	30·0				
6	0·0	18·0				
7	0·0	14·0				
8	0·0	8·0				
9	0·0	4·0				
10	0·0	0·0				

The group consisted of 6 rats of body-weights ranging from 100 g. to 140 g. They were fed on the basal diet including about 0.15 g. of fresh amaranth per rat per day. The level of carotene intake was about 13.0 γ per rat per day.

TABLE II—*contd.*3. *The absorption of carotene from amaranth (fat-rich diet).*

Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of carotene excreted through the faeces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	120.0	38.3	564.0	199.0	35.3	64.7
2	120.0	22.0				
3	120.0	28.6				
4	96.0	30.0				
5	108.0	24.2				
6	0.0	20.0				
7	0.0	12.3				
8	0.0	11.0				
9	0.0	7.6				
10	0.0	5.0				
11	0.0	0.0				
12	0.0	0.0				

The group consisted of 6 rats of body-weights ranging from 130 g. to 180 g. They were given a small quantity of amaranth + basal diet + 2 g. of coco-nut oil per rat per day. The fat content of the diet was about 30 to 40 per cent. The level of carotene intake was about 19.0 γ per rat per day.

TABLE II—*contd.*4. *The absorption of carotene from a mixed vegetable diet (diet No. 1) in groups of rats of different body-weights.*

Description.	Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of carotene excreted through the faeces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Group I: 3 rats, body-weights 40 g. to 50 g. Level of carotene intake about 13.0 γ per rat per day.	1	51.0	16.7	191.0	94.3	49.4	50.6
	2	23.0	19.0				
	3	39.0	21.0				
	4	39.0	12.6				
	5	39.0	10.0				
	6	0.0	6.0				
	7	0.0	5.0				
	8	0.0	..				
	9	0.0	4.0				
	10	0.0	0.0				
Group II: 3 rats, body-weights 100 g. to 120 g. Level of carotene intake about 19.0 γ per rat per day.	1	76.5	22.0	289.5	137.6	47.5	52.5
	2	39.0	43.0				
	3	58.0	20.6				
	4	58.0	17.0				
	5	58.0	10.0				
	6	0.0	8.0				
	7	0.0	10.0				
	8	0.0	..				
	9	0.0	7.0				
	10	0.0	0.0				

The higher intake in Group II was due to the consumption of larger quantities of food.

TABLE II—*contd.*5. *The absorption of carotene from red-palm oil (fat-free diet).*

Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of carotene excreted through the faeces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	78.2	16.5	330.8	136.8	41.4	58.6
2	96.0	30.0				
3	64.2	28.0				
4	54.0	24.3				
5	38.4	18.0				
6	0.0	9.5				
7	0.0	..				
8	0.0	6.5				
9	0.0	4.0				
10	0.0	0.0				

The group consisted of 6 rats of body-weights ranging from 110 g. to 140 g. They were supplied with sufficient quantities of fat-free basal diet + 1 drop of

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red-palm oil per rat per day (fed by hand). The level of carotene intake was about 11.0 γ per rat per day. The faeces of 7th and 8th days were extracted together.

TABLE II—concl'd.

6. *The absorption of carotene from red-palm oil in groups of rats of different body-weights.*

Description.	Days.	Quantity of carotene fed daily to the whole group of rats (γ) (0.001 mg.).	Quantity of carotene excreted through the faeces (γ).	Total quantity of carotene fed (γ).	Total quantity of carotene excreted (γ).	Percentage of carotene excreted.	Percentage of carotene retained in the body (from columns 4 and 5).
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Group I: 3 rats body-weights 40 g. to 50 g. Fed with a single dose of carotene = 117.0 γ per rat.	1	351.0	108.0	351.0	150.0	42.7	57.3
	2	0.0	29.0				
	3	0.0	10.0				
	4	0.0	3.0				
	5	0.0	0.0				
	6	0.0	0.0				
	7	0.0	0.0				
Group II: 3 rats, body-weights 100 g. to 120 g. Fed with a single dose of carotene = 117.0 γ per rat.	1	351.0	96.0	351.0	155.0	44.2	55.8
	2	0.0	39.0				
	3	0.0	13.0				
	4	0.0	5.0				
	6	0.0	2.0				
	7	0.0	0.0				
	8	0.0	0.0				

TABLE III.
The absorption of vitamin A and carotene from various foods.

Experiment number.	Nature of the food and the method of supply.	Number of experimental rats.	Daily dose of vitamin A and carotene per rat.	Total quantity of vitamin A or carotene fed to the whole group (γ).	Total quantity of vitamin A or carotene excreted by the whole group (γ).	Percentage of vitamin A or carotene excreted.	Percentage of vitamin A or carotene retained in the body (from columns 5 and 6).
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	Basal diet without yeast (deficient in vitamin-B complex), red-palm oil fed by hand in a single dose.	3	120.0	360.0 carotene.	154.5 carotene.	43.0 carotene.	57.0 carotene.
2	Basal diet with red-palm oil fed by hand in a single dose.	3	120.0	360.0 "	164.0 "	45.6 "	54.4 "
3	Red-palm oil fed by hand in a single dose. Basal diet with 2 g. of coco-nut oil per rat per day.	2	60.0	180.0 "	70.0 "	38.9 "	61.1 "
4	Red-palm oil fed by hand in a single dose. Basal diet without salt mixture.	3	120.0	360.0 "	167.0 "	46.4 "	53.6 "
5	Hallibut-liver oil fed by hand in a single dose, basal diet.	3	230.0	690.0 vitamin A.	26.2 vitamin A.	3.8 vitamin A.	96.2 vitamin A.

TABLE III—*concl'd.*

Experiment number.	Nature of the food and the method of supply.	Number of experimental rats.	Daily dose of vitamin A and carotene (γ) per rat.	Total quantity of vitamin A or carotene fed to the whole group (γ).	Total quantity of vitamin A or carotene excreted by the whole group (γ).	Percentage of vitamin A or carotene excreted.	Percentage of vitamin A or carotene retained in the body (from columns 5 and 6).
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
6	Egg-yolk mixed with basal diet; fed for 3 days.	6	17.0 6.0	306.0 carotene. 108.0 vitamin A.	118.0 carotene. 3.0 vitamin A.	38.6 carotene. 2.8 vitamin A.	61.4 carotene. 97.2 vitamin A.
7	Amaranth mixed with basal diet without fat; fed for 3 days.	6	32.5	585.0 carotene.	301.0 carotene.	51.5 carotene.	48.5 carotene.
8	Mixed diet No. 2; fed for 3 days.	6	60.0	1,080.0 "	435.0 "	40.3 "	59.7 "
9	Papaya mixed with basal diet; fed in a single dose.	8	26.0	208.0 "	90.0 "	43.3 "	56.7 "
10	Basal diet + orange juice fed by hand in a single dose.	8	10.0	80.0 "	33.0 "	41.2 "	58.8 "
11	Basal diet with very small amount of carotene; fed by hand in coco-nut oil in a single dose.	16	1.0	16.0 "	6.0 "	37.5 "	62.5 "

The feces passed during 5 days in the experiments Nos. 1, 2, 3, 4, 5, 9, 10, and 11 and during 8 days in the experiments Nos. 6, 7; and 8, after the first feeding, were collected together and examined as a single test material.

DISCUSSION.

From Tables I and II, it is seen that excretion of carotene in the faeces continued for 4 to 5 days, while that of vitamin A ceased within 2 to 3 days, after the supply of carotene or vitamin A respectively was stopped. Vitamin A was not detected in the faeces of carotene-fed animals. It was also observed that during the period in which vitamin-A depletion of the liver of rats was taking place, no faecal excretion of vitamin A occurred. It follows that faecal loss of vitamin A and carotene is due merely to incomplete digestion; the liver stores do not contribute to the faecal excretion. Depletion of the vitamin-A reserve, when the diet is deficient in vitamin-A activity, must be due solely to oxidation or some chemical transformations occurring within the tissues.

The data set out in Tables I and III (columns 5 and 6) show that only very little, 3 to 5 per cent, of vitamin A ingested (contained in halibut-liver oil, cod-liver oil, butter, and egg-yolk) was recovered from the faeces when the animals were fed at a level of 6γ to 230γ per rat per day. Baumann and Steenbock (1934) found that only a small fraction of vitamin A ingested could be recovered from the liver and faeces. They conclude that the major portion of vitamin A ingested is destroyed in the digestive tract. It is probable that some part of the unexcreted carotene will also be destroyed in the intestinal tract; precise knowledge on this question is, however, still lacking.

Excretion of carotene fed in amaranth, orange juice, papaya, egg-yolk, and the mixed diets, under varying conditions, ranged from 39 to 55 per cent (average 46 per cent) of the intake (Tables II and III), when fed at the daily levels of 10γ to 60γ . The faecal loss of carotene was found to be considerably greater when carotene was fed (in amaranth) in relatively large amounts—about 200γ per rat per day. Excretion of carotene when fed in red-palm oil, under varying conditions (Tables II and III), ranged from 37 to 47 per cent (average 42.5 per cent), when supplied at a level of 1γ to 120γ per rat per day. It is interesting to note that complete absorption of carotene never occurred, and even at the minimum level (1.0γ) of intake, 37.5 per cent was found to be excreted in the faeces. From Table II (column 1) and Table III (columns 1, 2, and 4) it appears that large amounts of carotene are probably better utilized when fed in oil solutions than when contained in natural foods, such as amaranth. The fat content of a diet, within the limits of 0 to 40 per cent, did not cause any significant difference in the absorption of vitamin A and carotene. An exception to this was noted in one case—Table II (column 3): absorption of carotene from amaranth was increased by about 10 per cent when the amaranth was fed with the basal diet containing about 30 to 40 per cent of fat.

Experiments Nos. 1, 2, 3, and 4, Table III, show that the absence of the vitamin-B complex (yeast) and the salt mixture, from a diet, does not materially influence the retention of carotene; so also retention of carotene was unaffected (Table II, columns 5 and 6) by the difference in body-weights of the experimental animals.

The above observations refer only to healthy and normal animals. The possibility, however, remains that absorption of vitamin A and carotene may be affected by various factors in the presence of pathological symptoms. Recently,

there have been several reports (Ralli *et al.*, 1935 ; Heymann, 1936*a* and *b* ; Wendt, 1936 ; and other workers), which show that the metabolism of both vitamin A and carotene may be affected in the absence of bile, in diabetes, hyperthyroidism, and in various infectious diseases.

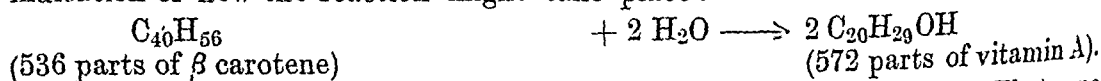
It may be noted that in the light of Clausen's (*loc. cit.*), and Baumann and Steenbock's (1934) work, the results of the present experiment on rats may be applicable to human beings.

A NOTE ON THE CONVERSION FACTOR I. U./E.

Carr and Jewell (1933) and Karrer and Morf (1933) have been able to isolate small quantities of vitamin A, in very pure forms, and their preparations showed $E \frac{1 \text{ per cent}}{1 \text{ cm.}}$ 3,280 A. U.=1,600 and 1,700 respectively. With the help of this data, spectrophotometric assays of vitamin A have become quantitative and the vitamin-A content of a test material can be expressed in absolute quantities. It is, however, desirable that vitamin-A values, estimated in absolute quantities, should be convertible into the customary unit, namely, the International Unit of vitamin A, which has been defined as the vitamin-A activity of 0.6 γ of pure β carotene. With a view to discovering the value of the conversion factor (I. U./E.), simultaneous biological and spectrophotometric studies were carried out, in different laboratories, on several given samples of cod-liver oil (Medical Research Council, 1935). The results varied widely, between 750 to 2,380.

The Vitamin-A Sub-Committee and the International Vitamin Conference, League of Nations, Health Organization (1934), on scrutinizing all the available data, have recommended the figure I. U./E.= 1,600 to be adopted provisionally, as a means of converting spectrographic values into International Units of vitamin A. From the figures, viz., $E \frac{1 \text{ per cent}}{1 \text{ cm.}} = 1,600$ or 1,700 for pure vitamin A, and I. U./E. = 1,600, it follows that one gramme of pure vitamin A will contain 256 to 272,000 I. U. ; or, in other words, 0.37 γ to 0.39 γ of vitamin A is equivalent to one I. U. (0.6 γ of pure β carotene). It thus appears that pure vitamin A is about 1½ times more potent than pure β carotene, a deduction contrary to theoretical considerations.

In the *in vivo* transformation of carotene into vitamin A, the following is an indication of how the reaction might take place :—



Therefore 1 γ of β carotene is converted into 1.067 γ of vitamin A. That one molecule of β carotene may be converted into two molecules of vitamin A is substantiated by the fact that, according to the molecular structure, one molecule of α carotene can give rise to only one molecule of vitamin A and the α isomer is half as potent as the β form. Thus theoretically, equal quantities of vitamin A and β carotene should possess very nearly the same degree of biological potency. This truth has to some extent been experimentally realized by Moore (1933) and Morgan *et al.* (1935) from feeding experiments on rats.

The discrepancy between the relative biological values of vitamin A and carotene, as described in the previous paragraph, and the relative values deduced

from their chemical nature, has so far escaped attention. An explanation for this discrepancy can now be given. In the present study on the absorption of vitamin A and carotene in rats, it has been noted that faecal loss of vitamin A is negligible, while about 40 per cent of carotene ingested (in oil solution) is eliminated in the faeces. The faecal loss of carotene appears to be practically constant even when carotene is supplied at a minimum level of 1 γ per rat per day. If we assume that the unexcreted fractions of vitamin A and carotene are equally affected by destruction taking place in the digestive tract, the following deductions are justified : out of 0.6 γ of β carotene ingested, $\frac{0.6 \times 40}{100} = 0.24\gamma$ will be excreted in the faeces, and 0.36 γ will be retained in the body. As a result of *in vivo* transformation according to the reaction noted above, the unexcreted portion of β carotene, on absorption, will give rise to $0.36 \times 1.067 = 0.38\gamma$ of vitamin A, an amount equal to that of vitamin A representing one I. U. as calculated above.

The factor I. U./E.=1,600, adopted provisionally, fits in quite harmoniously with the result of these absorption experiments and with the theoretical considerations, and hence, this value of the conversion factor appears to be the most appropriate. We can, therefore, conclude that the wide range of values (750 to 2,380), recorded for the ratio I. U./E., by various workers, is due rather to errors inherent in biological experiments than to any real variation in the ratio.

SUMMARY.

1. The relative utilization of vitamin A and carotene contained in a number of natural foods, namely, halibut-liver oil, cod-liver oil, butter, egg-yolk, red-palm oil, orange juice, papaya, amaranth, and two mixed diets, was investigated. The difference between the amount of vitamin A or carotene ingested in foods and that excreted in the faeces was taken as a measure of the amount absorbed in the body. 'Absorption', in this sense, includes the amounts of vitamin A or carotene that are destroyed in the digestive tract. Under varying conditions of feeding, absorption of vitamin A was found to be almost complete, while only 45 to 65 per cent of carotene ingested was retained in the body. It is thus clear that a considerable amount of carotene ingested is lost due to incomplete digestion and hence the biological value of vitamin A is much higher than that of its precursor, carotene.

2. The difference in the body-weights of experimental animals, and the fat, yeast (vitamin-B complex) and mineral content (salt mixture) of the diets did not cause any significant variation in the absorption of carotene. Vitamin A was found to be equally well absorbed from fat-free and fat-rich diets. It also appeared that large doses of carotene are better utilized when fed in an oil solution (red-palm oil) than in natural foods, such as amaranth.

3. The absorption experiments have provided some evidence in support of the appropriateness of the conversion factor I. U./E.=1,600, which was provisionally adopted by the International Vitamin Conference.

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THE RELATIVE VALUE OF THE PROTEINS OF CERTAIN FOODSTUFFS IN NUTRITION.

BY

M. SWAMINATHAN.

(*Nutrition Research Laboratories, I. R. F. A., Coonoor, S. India.*)

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A SYSTEMATIC examination of the relative biological values of the proteins of foodstuffs was undertaken for reasons given below. The comparative biological value of the proteins of 13 foodstuffs, including 5 cereals—raw milled rice, whole wheat, cambu, cholam, and ragi; 3 pulses—lentil, green gram, and soya bean; and 5 vegetables—drumstick leaves, amaranth leaves, ipomœa leaves, lady's fingers, and brinjal, have been studied by the nitrogen-balance method.

The biological value of the proteins of foodstuffs and isolated proteins has been the subject of numerous investigations in the past. Reference may be made to the works of McCollum and Simmonds (1929), Mitchell (1924*a*), Mitchell and Hamilton (1929), and Boas-Fixsen (1935) which provide reviews of the voluminous literature. Study of the biological value of vegetable proteins has been largely confined to the proteins of cereals and pulses, and only recently has attention been given to the proteins of vegetables (Adolph *et al.*, 1935). Further, the relative biological value of the proteins of various cereals and pulses is still a matter of uncertainty as the available data are conflicting.

McCay (1912) investigated various Indian foodstuffs and worked out the 'co-efficient of absorption' of their proteins by deducting protein excreted in the faeces from the protein intake. He assigned a value of 70.3 for the total proteins of wheat, 85.6 for green gram, 49.4 for cambu, and 53.6 for cholam. From his long series of experiments on various other foodstuffs he concluded that 'it is a mere truism to state that the gross chemical value of a food material may not necessarily be a measure of its real nutritive value and that the real value depends on the degree of absorbability of its proximate principles'.

Later, McCollum and Simmonds (1917) found wheat, maize, and rice to be of equal value for the maintenance of body-weight in rats and inferior to oats and millet; but from the results of other long term experiments (McCollum and Simmonds, 1921), they arranged the cereal proteins in the following descending order of merit: wheat, barley, rye, maize, and oats. Osborne and Mendel (1918) found that rice and barley supported growth better than did oats and maize. Sherman and his co-workers (1918 to 1920) found wheat, maize, and oats of approximately equal value in supplying the maintenance requirements of adult human

beings. Neither Mitchell and Carman (1924, 1926) nor Boas-Fixsen and her co-workers (1932, 1934), using the balance-sheet method, found any difference between the biological value of the proteins of wheat and maize. Niyogi *et al.* (1932) reported a biological value of 58 for lentil and 60 for green gram, while Basu *et al.* (1936) obtained a value of 32 for lentil and 52 for green gram, the value (58) obtained for lentil by the former group of workers being nearly twice that (32) obtained by the latter group of workers, though the level of protein intake was the same in all these experiments.

MATERIALS USED AND THEIR COMPOSITION.

The cereals and pulses used in the experiments were ground to fine powder. In the case of vegetables, the edible portions were cut into small pieces, dried first in the open air and finally in an air oven at a temperature of 40°C. to 50°C., after which the dried materials were ground to fine powder. Drying was necessary in the case of vegetables, as in the fresh state vegetables contain about 90 per cent of water, while their protein content varies from 1.5 to 6 per cent. It was, therefore, impossible to use the fresh materials to prepare diets containing 5 per cent of vegetable protein without the bulk of the diet becoming too great. Moisture was determined by drying samples to constant weight in an air oven at 100°C. and the 'crude protein' content ($N \times 6.25$) by the Kjeldahl method. The moisture and 'crude protein' content of the materials used as sources of protein are given in Table I:—

TABLE I.
Moisture and 'crude protein' content of test materials.

Name of foodstuff.	Botanical name.	Moisture per cent.	'Crude protein' per cent.
Raw milled rice	<i>Oryza sativa</i>	12.50	6.88
Whole wheat	<i>Triticum vulgare</i>	12.31	12.62
Cambu	<i>Pennisetum typhoideum</i>	12.10	10.48
Cholam	<i>Sorghum vulgare</i>	11.20	10.27
Ragi	<i>Eleusine coracana</i>	12.58	7.12
Lentil	<i>Lens esculenta</i>	12.44	25.70
Green gram	<i>Phaseolus radiatus</i>	10.42	23.80
Soya bean	<i>Glycine hispida</i>	9.30	40.00
Drumstick leaves (dry), powder (air dry).	<i>Moringa oleifera</i>	10.68	26.85
Drumstick „ (fresh)	„	(75.00)	(6.65)
Amaranth leaves powder (air dry)	<i>Amaranthus gangeticus</i>	11.20	29.05

TABLE I—concl'd.

Name of foodstuff.	Botanical name.	Moisture per cent.	'Crude protein' per cent.
Amaranth leaves powder (fresh) ..	<i>Amaranthus gangeticus</i> ..	(85·80)	(4·90)
Ipomœa leaves powder (air dry)	<i>Ipomœa reptans</i> ..	10·82	29·33
„ „ (fresh) ..	„ ..	(90·40)	(2·90)
Lady's fingers powder (air dry)	<i>Hibiscus esculentus</i> ..	10·51	14·94
„ „ (fresh) ..	„ ..	(88·00)	(2·20)
Brinjal powder (dry) ..	<i>Solanum melongena</i> ..	10·94	14·03
„ „ (fresh) ..	„ ..	(91·50)	(1·31)

TECHNIQUE AND FORMULÆ USED.

The technique employed is similar to that used by Chick *et al.* (1935*a* and *b*). Martin and Robison (1922), Mitchell (1924*b*), and Chick *et al.* (*loc. cit.*) have fully discussed the various problems involved in the calculation of the biological value and digestibility co-efficient, from the data obtained by the nitrogen-balance method.

The formulæ used by Chick and her co-workers, which have been adopted here, are as follows:—

I. The relative 'biological value' (B.V.) of a protein (p) is expressed as:—

$$100 \times \frac{\text{Body N saved}}{\text{Food N absorbed}} \quad \text{i.e., } 100 \left[\frac{U_p - U_e}{I_p - (F_p - F_e)} \right]$$

where I_p , U_p , and F_p are the daily N intake, urinary N excretion, and faecal N excretion respectively on a diet containing protein, and U_e and F_e are the daily endogenous N excreted in urine and faeces respectively on a nitrogen-free diet; $F_p - F_e$ represents the nitrogen in the faeces derived from undigested food protein and $I_p - (F_p - F_e)$ the true N intake on a diet containing protein.

II. The relative 'digestibility co-efficient' (D.C.) of a given protein (p) is expressed as:—

$$100 \left[\frac{\text{Food N digested}}{\text{Food N intake}} \right] \quad \text{i.e., } 100 \left[1 - \frac{(F_p - F_e)}{I_p} \right]$$

EXPERIMENTAL.

A group of 5 male rats (weighing about 150 g. at the commencement of the experiment) was used. The weights of the rats increased during the period of experiment as is shown in the figures given in Table III. It does not seem likely that the relatively small differences in weight of the rats in the various experiments would materially affect the comparability of the metabolism data obtained. The experimental diets containing the test materials and the nitrogen-free diet are shown in Table II:—

TABLE II.

Composition of the diets used.

	Nitrogen free g.	Milled raw rice g.	Whole wheat g.	Cambu g.	Cholam g.	Ragi g.	Lentil g.	Green gram g.	Soya bean g.	Drum- stick leaves g.	Amar- anth leaves g.	Ipomoea leaves g.	Lady's fingers g.	Brinjal g.
Foodstuff tested	..	75.0	42.0	50.0	48.0	70.0	42.0	44.0	25.0	19.0	17.0	17.0	33.3	35.6
Starch ..	71.2	..	32.0	26.0	26.0	8.0	33.0	31.0	50.0	53.0	53.0	55.0	40.0	38.0
Sugar ..	8.0	13.0	10.0	10.0	12.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Beef dripping ..	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	10.0	13.0	13.0	13.0	13.0	13.0
Salt mixture ..	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cod-liver oil ..	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calcium carbonate	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Percentage of N (dry weight).	0.0092	0.8192	0.8480	0.8560	0.8730	0.8624	1.7280	1.7120	1.0768	0.8800	0.8400	0.8403	0.8630	0.8736
* Crude protein* N x 6.25 dry weight.	..	5.12	5.30	5.35	5.46	5.39	10.80	10.70	10.48	5.50	5.25	5.25	5.39	5.46

Before the first test diet was given the rats received the nitrogen-free diet for 7 days; feeding with this diet was repeated at various intervals, usually after three-weekly test periods. The test diets were fed for a period of 7 days, in the order indicated in Table III. The first 3 days of each period is regarded as preparatory, allowing the rats to accommodate themselves to the new diets; the collection of urine and faeces was confined to the remaining 4 days of each period. The rats were given 'rest' for 4 days after each experimental period with the nitrogen-free diet as well as with the test diets. During the 'rest' period they received the stock diet. The level of protein intake was kept at approximately 5 per cent in the diets containing the cereals and vegetables and at 10 per cent in those containing pulses, since the protein content of the former is relatively lower. Results tend to be higher at a low level of protein intake. The figures obtained will, however, serve for drawing comparisons among the cereals and vegetables themselves.

The experimental diets were prepared by adding the necessary ingredients in the desired proportions, as shown in Table II. In addition, 3 c.c. of an aqueous solution of yeast extract (corresponding to 0.75 g. of dry yeast) containing the vitamin-B complex were given daily to each rat along with food. The diets were thoroughly mixed up, made into a paste by adding distilled water in order to prevent scattering of food, and samples were analysed for their moisture and protein content. The dry weight of the diets before the addition of water was noted and also the wet weight in the pasty condition. In weighing out the daily food, care was taken that the food given should not greatly exceed the average food consumption, thus avoiding a large residue. Food residue remaining uneaten was carefully collected daily, completely dried in an air oven at 100°C., and corresponding weights of the residual food subtracted from the dry weight of the food given daily to the rats. By this means daily food intake was obtained.

The urine and faeces were collected daily. To prevent decomposition of urine and consequent loss of ammonia, 20 c.c. of 5 per cent dilute sulphuric acid containing 2 per cent of carbolic acid and 1 per cent of thymol were placed in the flasks used to collect urine. The metabolism cages, funnels, separators, and flasks placed for collection of urine were washed down daily with small quantities of distilled water and the washings were added to the urine bottle. At the end of each experiment the urine and washings were filtered through muslin into a 500 c.c. standard flask, the volume made up to mark and then transferred into a clean stoppered bottle. Aliquot portions were removed for nitrogen estimation. The faeces were transferred daily into a small wide-mouthed bottle provided with a lid. At the end of each experiment the faeces were moistened with 10 c.c. of 5 per cent oxalic acid, dried in an air oven at 100°C. for a number of hours, cooled and then powdered in a glass mortar. The powdered faeces were then transferred to a small wide-mouthed weighed bottle, dried again at 100°C. and the weight again determined. The difference between the two weights gave the weight of the faeces. Aliquot portions were weighed out for nitrogen estimation. All the nitrogen estimations were done by the Kjeldahl method. In the calculation of the biological values and digestibility co-efficients, the average of the figures obtained for the endogenous urinary and faecal nitrogen at the beginning and end of a group of experiments was taken for that group, since no regular variations were observed in their value.

The data regarding the metabolism experiments are given in Table III:—

TABLE III.

(Figures of intake and excretion represent daily averages.)

Rat number.	Initial body-weight g.	Final body-weight g.	Food intake g.	N intake mg.	Urinary N mg.	Faecal N mg.	Body N saved mg.	Food N absorbed mg.	Biological value per cent.	Digestibility co-efficient per cent.
1	138	133	11.90	..	33.5	16.0	..	..	..	..
2	152	145	15.70	..	44.9	19.5	..	..	..	..
3	144	139	11.80	..	36.3	17.1	..	..	..	..
4	145	141	11.30	..	37.7	16.6	..	..	..	..
5	131	128	10.70	..	33.2	11.5	..	..	..	..

Period 2.—Milled raw-rice diet $N = (0.8192)$ per cent.										
1	139	142	12.76	104.6	53.2	19.2	82.7	102.3	81	98
2	155	163	16.36	134.2	78.8	25.6	92.6	128.7	72	96
3	146	150	12.18	99.9	48.8	19.4	83.2	96.6	86	97
4	145	149	11.82	96.9	58.5	19.1	73.2	92.9	79	96
5	132	134	9.74	79.7	45.0	15.3	63.2	77.8	81	98
Average ..									80	97

TABLE III—*contd.**(Figures of intake and excretion represent daily averages.)*

Rat number.	Initial body-weight g.	Final body-weight g.	Food intake g.	N intake mg.	Urinary N mg.	Faecal N mg.	Body N saved mg.	Food N absorbed mg.	Biological value per cent.	Digestibility co-efficient per cent.
<i>Period 9.—Green-gram diet N = (1.7120) per cent.</i>										
1	184	183	13.13	224.8	125.4	48.0	108.4	196.3	55	55
2	214	217	15.52	265.7	159.4	54.1	116.5	232.0	50	87
3	182	193	14.11	241.5	140.0	56.8	96.8	201.3	48	83
4	193	191	12.81	219.3	135.2	46.3	91.5	188.0	49	86
5	174	175	11.33	194.0	112.8	43.1	90.0	166.8	54	86
								Average ..	51	86
<i>Period 10.—Soya-bean diet N = (1.6768) per cent.</i>										
1	192	195	13.33	223.9	110.4	73.8	96.7	169.6	57	76
2	228	222	12.65	212.5	118.8	49.1	108.9	183.8	59	85
3	198	196	10.98	184.5	100.5	69.0	67.2	132.1	51	72
4	179	179	11.18	187.8	103.9	58.4	79.2	144.4	55	77
5	181	180	9.73	163.5	94.8	62.0	58.6	117.4	50	72
								Average ..	54	76

TABLE III—*contd.*

(Figures of intake and excretion represent daily averages.)

Rat number.	Initial body-weight g.	Final body-weight g.	Food intake g.	N intake mg.	Urinary N mg.	Faecal N mg.	Body N saved mg.	Food N absorbed mg.	Biological value per cent.	Digestibility co-efficient per cent.
<i>Period 15.—Nitrogen-free diet N = (0.0092) per cent.</i>										
1	222	215	14.10	..	42.0	22.7	..	..	..	..
2	240	237	15.20	..	47.8	25.8	..	..	..	..
3	180	179	12.95	..	31.9	19.4	..	..	..	..
4	190	185	12.78	..	44.1	16.8	..	..	..	..
5	192	184	12.43	..	41.3	18.4	..	..	..	..

Period 16.—Lady's fingers diet N = (0.8630) per cent.

1	223	213	16.03	138.3	58.8	57.3	87.1	103.3	84	75
2	246	238	16.73	144.5	67.4	63.2	85.7	106.0	81	73
3	186	187	11.93	103.1	50.6	51.8	55.4	71.3	78	69
4	202	193	11.23	97.0	52.2	50.1	53.9	63.9	84	67
5	195	188	15.10	130.5	56.7	59.3	74.5	89.2	84	68
Average ..									82	70

TABLE III—*concl.**(Figures of intake and excretion represent daily averages.)*

Rat number.	Initial body-weight g.	Final body-weight g.	Food intake g.	N intake mg.	Urinary N mg.	Faecal N mg.	Body N saved mg.	Food N absorbed mg.	Biological value per cent.	Digestibility co-efficient per cent.
<i>Period 17.—Brinjal diet N = (0.8736) per cent.</i>										
1	221	218	13.43	116.8	70.4	49.9	61.4	89.2	67	76
2	246	235	14.30	124.4	71.6	52.5	72.1	96.6	75	78
3	189	181	10.20	88.7	55.3	40.3	47.8	68.4	70	77
4	195	186	11.45	99.6	61.3	45.5	52.0	71.1	73	71
5	191	187	12.03	104.7	64.4	47.2	53.1	75.5	70	72
Average ..									71	75

Period 18.—Nitrogen-free diet N = (0.0092) per cent.

1	224	213	14.80	..	43.2	21.8	..	..	..	..
2	244	231	15.50	..	46.4	23.6	..	..	..	..
3	191	186	12.85	..	37.5	20.5	..	..	..	..
4	193	187	12.55	..	40.2	17.2	..	..	..	..
5	197	191	14.20	..	42.7	17.2	..	..	..	..

DISCUSSION.

All the results reported in this paper relate to the total nitrogenous constituents of the foodstuffs examined, referred to as their 'crude protein' content. Many foodstuffs, e.g., vegetables, contain considerable amounts of non-protein nitrogen, part of which may exist as free amino acids, though in vegetables most of this is in the form of amides or amino acids, like asparagine, etc. The biological value of the proteins in a food may in most cases be enhanced by the supplementary effect of this non-protein nitrogen, consisting largely of the simpler, easily digestible, and assimilable peptides and amino acids and their derivatives (Grindley and Eckstein, 1916; Mitchell, 1924a; Bhagvat and Sreenivasaya, 1935).

Cereals.—There is no doubt that proteins of animal origin are as a class superior in biological value to those derived from plants. Cereal proteins, though inferior to animal proteins, nevertheless possess higher biological values than those of legumes. The proteins of rice are found to be distinctly superior in biological value to those of wheat, when the proteins are fed at the same level of intake. Wheat, however, contains nearly twice as much protein as rice. The biological values obtained, 80 for rice and 66 for wheat, agree well with the values reported by previous workers. Mitchell *et al.* (*loc. cit.*) reported a value of 86 for rice and 67 for wheat, while Boas-Fixsen *et al.* (*loc. cit.*) obtained a value of 68 for wheat. The proteins of cambu, cholam, and ragi possess higher biological values than those of wheat, but their digestibility co-efficients are lower. The proteins of cambu and cholam are of almost equal biological value (83) but the proteins of ragi, having a biological value of 89, appear to be the best of all cereal proteins for the maintenance of nitrogenous equilibrium in adult rats. The biological value (89) obtained for ragi proteins is in close agreement with the value (90.5) reported by Niyogi *et al.* (1934). On the basis of the present experiments, the cereal proteins range themselves as regards biological value in the following descending order: ragi, cholam, cambu, rice, and wheat.

Legumes.—Legumes in general contain a high percentage of protein and so form an important source of proteins in Indian diets; the proteins of legumes, however, were found to be the poorest in value of all those investigated, which is probably due to their deficiency in the essential amino acid cystine (Jones *et al.*, 1922, 1924). It was pointed out in the introduction that the biological values obtained by the previous workers for lentil and green gram are conflicting, Niyogi *et al.* (1934) reporting values of 58 and 60 and Basu *et al.* (*loc. cit.*) 32 and 52, respectively. The values obtained in the present investigation, 41 for the proteins of lentil and 51 for the proteins of green gram, agree more closely with those reported by Basu *et al.* The proteins of soya bean were found to be superior to those of green gram and lentil, and in this respect soya bean appears to resemble the nuts and oil-seeds, such as pea-nuts and coco-nuts, the biological values of whose proteins have been assessed at 58 and 58 respectively by Mitchell *et al.* (*loc. cit.*). The biological value (54.4) obtained for the proteins of soya bean in the present investigation is lower than that (64) reported by Mitchell *et al.* (*loc. cit.*). From the present experiments the pulse proteins tested may be arranged in the following descending order of merit as regards their biological value: soya bean, green gram, and lentil.

Vegetables.—Though vegetables do not contribute largely to the total proteins of the diet, they are in proportion to their calorie-yield surprisingly rich in protein.

On an air-dry basis, vegetables contain much more proteins than cereals and some of them contain as much protein as the pulses, e.g., amaranth leaves 29·05, ipomœa leaves 29·33, drumstick leaves 26·85, lady's fingers 14·94, and brinjal 14·03. Our information regarding the quality of the proteins in vegetables is scanty. Sherman (1933) remarks: 'That the dry legumes are both absolutely and relatively rich in protein is a fact so well recognized as not to require elaboration. Less generally realized is the fact that while the green vegetables contain too much water to show high absolute values or percentages by weight of protein, yet they show as much or more of the total fuel value in the form of protein as is customary or desirable in ordinary dietaries. The vegetables differ among themselves in the nutritive efficiency of their proteins. Probably the proteins of the leafy vegetables are, weight for weight, of somewhat higher nutritive value than the proteins of seeds. Even among the latter, there are also differences, which naturally have been studied mainly in the legumes, since they are so generally regarded as significant largely for their protein content'.

The substantial truth of these remarks is confirmed by the results of the present investigation and by the work of Adolph *et al.* (*loc. cit.*) on cabbage and sweet potato proteins. Of the five vegetables investigated, four possess proteins of high biological value: lady's fingers 82, amaranth 72, brinjal 71, and ipomœa leaves 67, which are thus superior in nutritive value to the proteins of legumes. The proteins of drumstick leaves, like those of lentil, are of low biological value (41). In experiments in which the level of protein intake was 10 per cent, Adolph *et al.* (*loc. cit.*) observed a biological value of 76 for the crude isolated proteins of cabbage leaves and a value of 72 for the crude isolated proteins of sweet potato. Mitchell *et al.* (*loc. cit.*) recorded a biological value of 67 for the total proteins of potato, which were fed at an 8 per cent level, while Kon (1928) obtained a value of 71 for 'tuberin', the chief protein of potato. From the above results it may be concluded that the proteins of most of the vegetables so far investigated are of high biological value and probably are of value in supplementing the proteins of cereals and pulses. The vegetable proteins investigated fall into the following descending order of merit as regards their biological value: lady's fingers, amaranth leaves, brinjal, ipomœa leaves, and drumstick leaves.

TABLE IV.

The available or net protein content of certain foodstuffs.

Name of foodstuff.			Moisture per cent.	'Crude protein' per cent.	Level of protein intake per cent.	Biological value per cent.	Digestibility co-efficient per cent.	Available or net protein per cent.
Raw polished rice	..		12·50	6·88	5	80	97	5·34
Wheat	..	..	12·31	12·62	5	66	93	7·75
Cambu	..	..	12·10	10·48	5	83	89	7·74
Cholam	..	..	11·20	10·27	5	83	91	7·76

TABLE IV—concl'd.

Name of foodstuff.	Moisture per cent.	'Crude protein' per cent.	Level of protein intake per cent.	Biological value per cent.	Digestibility co-efficient per cent.	Available or net protein per cent.
Ragi	12.58	7.12	5	89	80	5.07
Lentil	12.44	25.7	10	41	88	9.27
Green gram .. .	10.42	23.8	10	51	86	10.44
Soya bean	9.30	40.0	10	54	76	16.42
Drumstick leaves (dry) ..	10.68	26.85	5	41	77	8.48
„ „ (fresh) .	(75.00)	(6.65)	..	..	..	(2.72)
Amaranth leaves (dry) ..	11.20	29.05	5	72	78	16.31
„ „ (fresh) ..	(85.80)	(4.90)	..	..	..	(2.75)
Ipomœa leaves (dry) ..	10.82	29.33	5	67	85	16.70
„ „ (fresh) ..	(90.40)	(2.90)	..	..	..	(1.65)
Lady's fingers (dry) ..	10.51	14.94	5	82	70	8.58
„ (fresh) ..	(88.0)	(2.2)	..	..	..	(1.26)
Brinjal (dry)	10.94	14.03	5	71	75	7.47
„ (fresh)	(91.50)	(1.31)	..	..	..	(0.70)

THE 'AVAILABLE OR NET PROTEIN CONTENT' OF FOODSTUFFS.

The term 'available or net protein content' of a given foodstuff denotes the actual amount of protein utilized by the body for every 100 g. of foodstuff intake. For a complete determination of this figure, it is necessary to consider (1) the total nitrogen content of the foodstuff, i.e., the crude protein content ($N \times 6.25$), (2) the loss or wastage of protein nitrogen in metabolism, i.e., during the process of its conversion into tissue constituents or body secretions, and (3) the loss or wastage of protein nitrogen in digestion. 'The available or net protein content' of a foodstuff, therefore, depends as much upon its 'crude protein' content as upon the extent of its utilization in digestion and in anabolism, as shown by Mitchell and Beadles (1927). It may be calculated by the combined use of the above three factors, viz., the 'crude protein' content, the digestibility co-efficient, and the biological value. Mitchell's formula is as follows:—

'Available or net protein content' =

$$\text{'crude protein' per cent} \times \frac{\text{Digestibility co-efficient}}{100} \times \frac{\text{Biological value}}{100}$$

The 'available protein content' of the 13 foodstuffs investigated is given in Table IV. Just for comparison, some data regarding the protein content, digestibility co-efficient, biological value, and 'available protein' or 'protein value' of some other foodstuffs are reproduced in Table V from Mitchell and Hamilton's book 'The Biochemistry of Amino Acids', p. 556.

TABLE V.

'Protein value' of foods form maintenance and growth on an 8 to 10 per cent level of protein intake (Mitchell and Hamilton, 1929).

Food.	Moisture.	PROTEIN CONTENT PER CENT.		Digestibility co-efficient per cent.	Biological value per cent.	AVAILABLE PROTEIN CONTENT PER CENT.	
		Fresh basis.	Dry basis.			Fresh basis.	Dry basis.
Whole egg ..	73.2	13.2	49.3	100	94	12.0	44.9
Milk ..	87.0	3.3	25.4	100	85	2.6	20.2
Beef liver ..	71.2	20.4	70.8	90	77	14.9	51.1
Rolled oats ..	7.7	16.7	18.1	90	65	9.8	10.6
Whole wheat ..	11.4	13.8	15.6	91	67	7.1	8.1
Whole corn ..	10.3	7.5	8.4	95	60	3.0	3.5
Potato ..	78.3	2.2	10.1	78	67	0.8	3.9
Navy beans ..	12.6	22.5	25.7	76	38	4.2	6.0
Cocoa ..	4.6	21.6	22.6	38	37	1.6	1.8

If results obtained with rats are applicable to human beings, the 'available or net protein' content of a foodstuff is of importance in planning diets. As a rule, the protein content of diets is calculated by consulting tables of chemical analysis of foodstuffs, which give 'crude protein' content. The possible error involved in such calculations may be illustrated as follows: lentil, which contains 25.7 per cent of 'crude protein' of which 88 per cent only is digestible, contains 22.4 per cent of digestible protein. Since only 41 per cent of the digestible protein can be used to cover the protein requirements of the body at a 10 per cent level of protein intake, lentil contains only 9.27 per cent of 'available or net protein'. Suppose a diet is planned which according to tables based on chemical analysis contains 25.7 g. of protein derived from lentil alone. Only 9.27 g. of that protein are available for utilization by the body and the remaining 16.43 g. of protein are wasted in digestion and metabolism. Green gram contains 23.8 per cent of 'crude protein' of which 86 per cent is digestible; of the latter only 51 per cent is available for body purposes, so that this food contains only 10.23 per cent of 'available protein'. Navy beans (Mitchell and Hamilton, *loc. cit.*), with 22.5 per cent of 'crude protein', contain only

4.2 per cent of 'available protein'. It is obvious, from the above examples, that in practical dietetics the quality of the proteins should be taken into consideration, as well as the quantity, since all proteins, especially proteins of vegetable origin, are not of the same quality.

It must be admitted that in basing the protein value of a foodstuff on its available or net protein content, no consideration is given to the supplementary relationships which may exist between different proteins in a diet and to the fact that different proteins may have different supplementary values.

SUMMARY.

1. The biological values and digestibility of the proteins of 13 foodstuffs, which include 5 cereals—rice, wheat, cambu, cholam, and ragi; 3 pulses—lentil, green gram, and soya bean; and 5 vegetables—drumstick leaves, amaranth leaves, ipomœa leaves, lady's fingers, and brinjal, have been determined by the balance-sheet method on adult male rats.

2. On a 5 per cent level of protein intake, the digestibility co-efficient and biological value of rice are 97 and 80, of wheat 93 and 66, of cambu 89 and 83, of cholam 91 and 83, and of ragi 80 and 89, respectively. By taking into consideration both the digestibility co-efficient and biological value, the cereal proteins may be arranged in the following descending order of merit: rice, cholam, cambu, ragi, and wheat.

3. At a 10 per cent level of protein intake the digestibility co-efficient and biological value of lentil are 88 and 41, of green gram 86 and 51, and of soya bean 76 and 54, respectively. Taking into consideration both the digestibility co-efficient and biological value, the pulse proteins may be arranged in the following descending order of merit: green gram, soya bean, and lentil.

4. At a 5 per cent level of protein intake, the digestibility co-efficient and biological value of the proteins of drumstick leaves are 77 and 41, of amaranth leaves 78 and 72, of ipomœa leaves 85 and 67, of lady's fingers 70 and 82, and of brinjals 75 and 71, respectively. On a similar basis the proteins of vegetables may be arranged in the following descending order of merit: lady's fingers, ipomœa leaves, amaranth leaves, brinjal, and drumstick leaves.

5. As regards 'available or net protein content', the cereals range themselves in the following descending order: cholam, wheat, cambu, rice, and ragi; the pulses: soya bean, green gram, and lentil, and the vegetables (in a fresh state): amaranth leaves, drumstick leaves, ipomœa leaves, lady's fingers, and brinjal.

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BALANCED DIETS.*

Part I.

BY

S. P. NIYOGI, M.Sc., M.B.,

V. N. PATWARDHAN, M.Sc., PH.D. (Lond.),

AND

R. G. CHITRE, M.Sc.

*(Department of Physiology, Seth Gordhandas Sunderdas Medical College,
Parel, Bombay.)*

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THE fact that the low standard of health among the bulk of the Indian population is not so much due to under-feeding as to ill-feeding has been widely acknowledged only recently. The problem of finding a cheap and complete dietary, paying due heed, of course, to the local conditions and habits, has been engaging the attention of scientific as well as social workers. The Bombay Presidency Baby and Health Week Association (1935) have been trying to disseminate some useful information concerning the value of foodstuffs in common use in this part of the country. The Association has also formulated from time to time certain diets to meet the requirements of an individual at a maximum monthly cost of Rs. 6 per adult at the market rate of the food constituents. These diets are called the 'balanced diets' and provision is made in these for the fat, protein, carbohydrate, and caloric requirements of a moderately hard-working individual. Diets at an average monthly cost of Rs. 7 are also included for those who can afford to spend more.

* The subject-matter of this paper forms a part of the investigations on the nutritive value of certain diets, which are carried out by a Committee under the joint auspices of the Bombay Presidency Baby and Health Week Association and the Seth Gordhandas Sunderdas Medical College, Bombay, consisting of Doctors H. V. Tilak, B.Sc., M.B., B.S., F.R.C.S.E., and K. S. Mhaskar, M.D., D.P.H., representing the Association, and of Doctors Jivraj N. Mehta, M.D. (Lond.), M.R.C.P. (Lond.), S. P. Niyogi, M.Sc., M.B. (Cal.), and V. N. Patwardhan, M.Sc., PH.D. (Lond.), representing the College. The Committee is also assisted by Dr. A. K. Boman-Behram, M.B., B.S., and Mr. R. G. Chitre, M.Sc. The Indian Research Fund Association and the Sir Dorabji Tata Memorial Trust have helped the investigation by substantial grants.

Some experiments conducted by the Association on the boys and girls at the Byramjee Jeejeebhoy Home, Matunga, Bombay, showed that those fed on the 'balanced diet' grew better than the others who were on the routine diet of the home. It was with a view to investigating scientifically the adequacy of this diet and improving upon it wherever necessary that the present investigation was undertaken. A complete study of the vegetarian and non-vegetarian diets would be necessary; the present paper represents the laboratory experiments on non-vegetarian diets only. The authorities of the David Sassoon Industrial School for boys, Matunga, Bombay (hereafter referred to as D. S. I. School), have extended their co-operation, making it possible to conduct controlled experiments on the inmates.

For purposes of comparison another diet has been evolved in the laboratory by taking into account the commonly accepted (Report of the Committee on Nutrition appointed by the British Medical Association, 1933) human requirements of fats, proteins, carbohydrates, calories, vitamins, and minerals. In the case of fats and proteins more attention has been paid to the inclusion in the diet of substances of animal origin in sufficient amounts. This diet has been named the 'physiologically ideal diet', its calculated cost at market rates amounts to Rs. 10 per month per adult. The 'routine diet' of the D. S. I. School, the cost of which at market rates is Rs. 4-8 per month per adult, served as control. In the following pages these diets will be referred to as P. I. D., B. D. and R. D.

Feeding experiments on the inmates of the D. S. I. School and the growth tests on albino rats were carried out simultaneously with the non-vegetarian diet. This communication deals with the animal experiments. The experiments on boys which are being conducted by Dr. K. S. Mhaskar and Dr. A. K. Boman-Behram will form the subject of a later communication.

Attempts to find cheap and well-balanced diets are progressing along somewhat similar lines in other parts of India. Wilson, Ahmad and Mullick (1936) have made a diet survey of some middle-class families and some institutions in Calcutta; they found that the cost of an adequate diet, per man value, per day, at Calcutta was between 4.4 and 5.6 annas entailing a monthly expenditure of Rs. 7 to Rs. 10 per head. Aykroyd and Krishnan (1936) have examined at Coonoor a number of cheap diets. They have investigated the effect of including in the diet the staple cereals like rice, wheat, jowar (*Sorghum vulgare*), bajri (*Pennisetum typhoides*), and ragi (*Eleusine coracana*) in various proportions supplemented by legumes, root and leafy vegetables, dried skim-milk, butter-milk, and jaggery. From among several cheap diets examined the one on which they obtained the best growth was constituted as follows:—

			Oz.
Ragi (<i>Eleusine coracana</i>)	..	..	16
Soya bean	..	..	2
Dhal arhar (<i>Cajanus indicus</i>)	..	..	2
Jaggery	..	..	1
Leafy vegetables	..	..	8
Root vegetables	..	..	2
Coco-nut oil	..	..	1.5
Butter-milk	..	..	6

The average weekly increase in weight of 12 young rats during 10 weeks on this diet was 9·8 g., the corresponding average for their stock diet being 10·6 g.

EXPERIMENTAL.

The diets.—In Table I is shown the composition of the diets per adult person under investigation. A few changes were made in the 'balanced diet' during the course of the investigation; the composition of both of these is given in the table:—

TABLE I. •

Article.	QUANTITY IN OUNCES.			
	P. I. D.	B. D.		R. D.
		Original.	Modified.	
Polished rice	6	10	9	8
Whole-wheat flour	6	6	8·5	7
Jowar (<i>S. vulgare</i>)	4	..	..	..
Bajri (<i>P. typhoideum</i>)	..	..	..	5
Pulses	1	1	1·7	4
Soya bean	..	1·5	1·5	1
Rice bran	..	1·0	1·0	..
Ground-nut oil cake	..	0·5	0·5	..
Vegetable oil	1	1	1	1
Mutton fat	1	1	1·3	..
Ghee	0·5	..	..	..
Dried skim-milk	2	0·5	1·5	..
Fresh whole milk	6	..	..	..
Eggs	1	..	..	..
Meat and fish	3	3	3	0·5
Sugar and jaggery	2	1·7	1·5	2
Root vegetables	8	5·5	3	10
Leafy vegetables	6	3·5	5·5	..
Lemons and tomatoes	4	..	..	..

The collection and analyses of the diets.—The diets were cooked in the Industrial School and brought over to the laboratory where they were dried at 40°C. to 45°C. in a current of hot air for about eight to ten hours. The material was then powdered and kept stoppered at room temperature. The diets thus collected were analysed from time to time to keep a check on the distribution and also to make certain that the analytical figures agreed with those calculated from the data given by McCarrison (1931). Table II shows the results of the actual analyses:—

TABLE II.

Analyses of the experimental diets showing the total quantities ingested per day per adult.

Constituent.	P. I. D.	B. D.		R. D.
		Original.	Modified.	
	g.	g.	g.	g.
Proteins, total	103.2	87.6	100.5	88.7
„ of animal origin	(51.6)	(29.7)	(36.9)	(4.2)
Fats, total	82.5	71.5	81.6	31.7
„ of animal origin	(52.5)	(30.8)	(40.2)	(0.4)
Carbohydrates	464	472	446	586
Crude fibre	11.0	12.1	14.3	14.9
Iron (Fe)	0.19	0.16	0.13	0.23
Calcium	2.57	1.43	1.74	2.55
Magnesium	1.11	1.16	1.08	1.2
Phosphoric acid (H_3PO_4)	6.57	6.33	6.66	5.1
Sulphuric acid (H_2SO_4)	2.89	1.77	1.53	1.65
Chlorides as NaCl	14.16	15.0	14.43	14.3
Insoluble matter	0.76	0.50	0.49	0.97
Total calorific value of the diet	3,000	2,960	3,000	3,060

The capacity of a protein or a mixture of proteins to promote growth and maintain an animal in health will be determined by its content of those amino acids which are indispensable for growth and maintenance. Evidence reviewed by Mitchell and Hamilton (1929) shows that lysine, cystine, and tryptophane can be considered as essential amino acids. The indispensability of tyrosine has not been proved beyond doubt. Arginine and histidine seem to be interconvertible in the animal body. The amounts of all the six, however, have been determined.

The essential amino acids were estimated after hydrolysis of the fat-free diet with hydrochloric acid (1:1). The mono-amino acids were separated from the di-amino acids by the precipitation of the latter with phosphotungstic acid by Hausmann's method as modified by Osborne and Harris (1903). The amounts of cystine, lysine, histidine, and arginine were determined according to van Slyke's (1915) method of nitrogen distribution in the phosphotungstic acid precipitate.

Tyrosine was determined in an aliquot portion of the original hydrolysate by the method of Folin and Denis (1912). Tryptophane was calculated on the basis of humin nitrogen obtained on acid hydrolysis (Gortner and Holmes, 1917).

TABLE III.

Essential amino acid content of the diets.

Amino acid.	P. I. D. per cent.	B. D. (original) per cent.	R. D. per cent.
Tyrosine ..	0.13	0.10	0.11
Arginine ..	0.80	0.72	0.72
Lysine ..	0.20	0.05	0.07
Histidine ..	0.68	0.87	0.78
Cystine ..	0.59	0.49	0.51
Tryptophane ..	0.51	0.64	1.10

Influence on the growth of rats.—Young growing rats from healthy litters four to five weeks old were selected for this experiment. Fifteen rats were kept on the P. I. D., fourteen on B. D., and eleven on R. D. To eliminate errors due to any

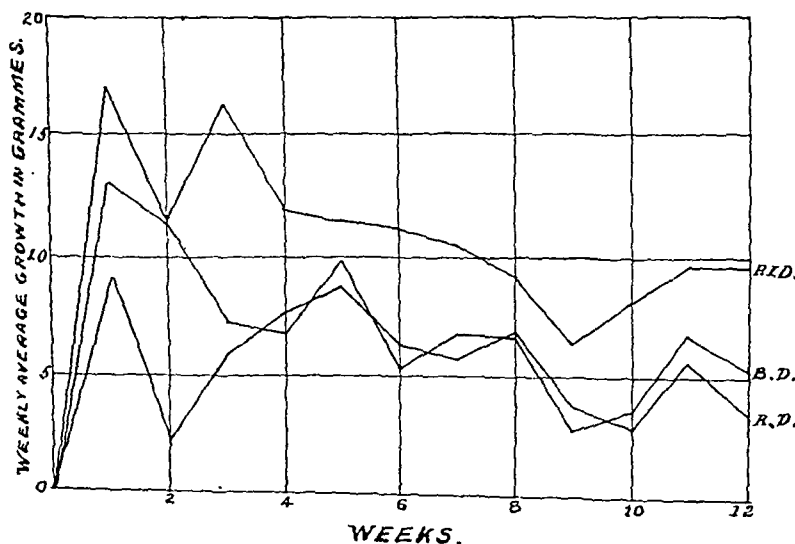


Fig. 1. Showing the average weekly increase in weights of rats on experimental diets.

similarity of response among litter mates the number in each group was made up by taking rats from different litters. Males and females were segregated. Food and water were given *ad lib*. Rats were weighed twice every week for a period of 12 weeks. In Fig. 1 is plotted the average weekly increase in weight in each group.

In Fig. 2 are shown the best growths obtained in a male and a female rat from each group.

At the end of March 1936 some alterations were made in the constituents of the B. D.; the modified diet is shown in Table I. The main alteration consisted of the addition of an extra ounce of skim-milk powder which required minor adjustments in other directions. Slight alterations were also made in the P. I. D. to make good the loss which occurred during the process of cooking. These alterations,

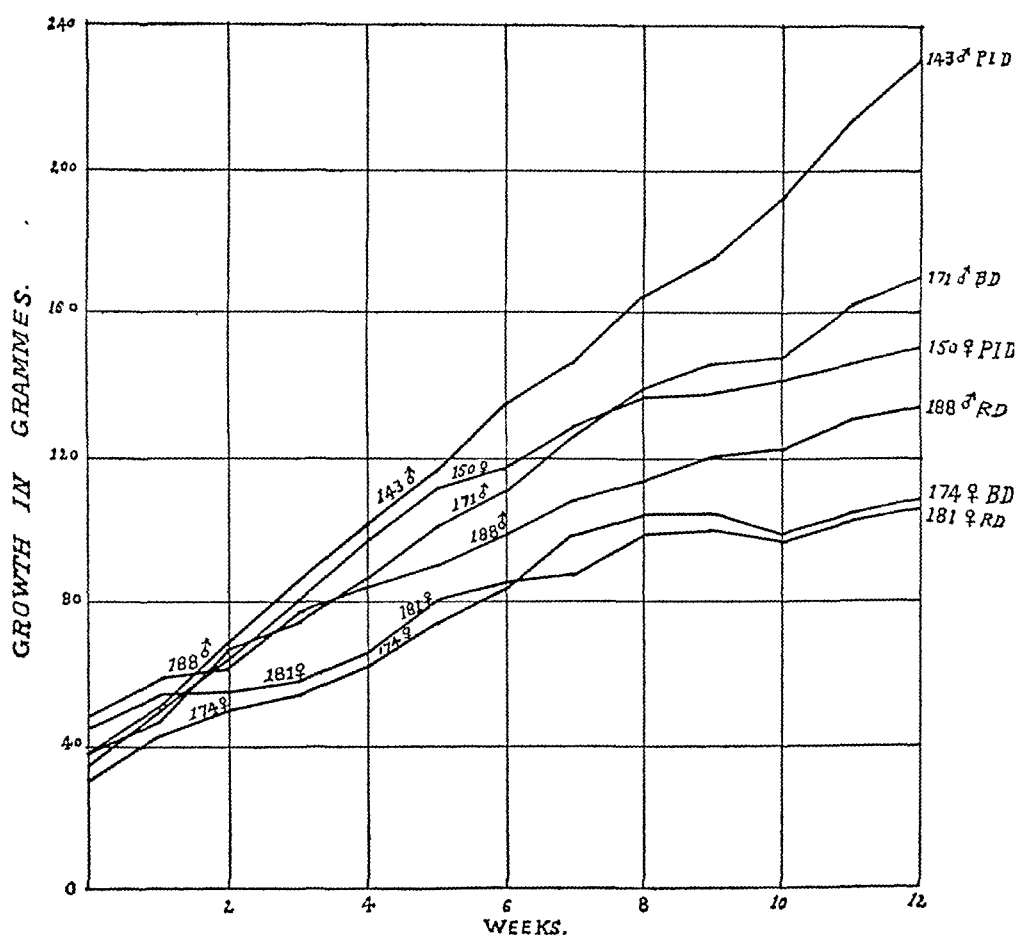


Fig. 2. Showing the maximum growth of a male and a female from each diet group.

however, did not materially affect the caloric value of the diet. Feeding experiments with B. D. on albino rats were started in three batches in early February and early March of 1936 with the result that all the rats belonging to the B. D. group had been on this modified diet for periods ranging from four to eight weeks. Reference will be made to this fact later.

Influence on reproduction.—When the rats had attained the age of 120 to 130 days males and females were brought together, one pair in each cage. The number of days elapsing before the arrival of the litters and the number of young rats in

each litter were noted. The mothers were separated from the young rats after three weeks and mating repeated with the same male and similar observations repeated. The results are given in Table IV:—

TABLE IV.

Influence of diet on the reproductive ability of rats.

Diet.	Serial number of female rat.	NUMBER OF DAYS FROM MATING TO BIRTH OF THE LITTER.		NUMBER OF YOUNG RATS IN THE LITTER.	
		1st.	2nd.	1st.	2nd.
P. I. D. ..	146	25	24	7	11
.. ..	144	24	..	5	..
.. ..	148	26	24	9	4
.. ..	191	25	25	7	9
.. ..	192	24	28	6	10
B. D. ..	174	23	25	4	3
.. ..	183	39	..	6	..
.. ..	196	24	32	6	4
.. ..	198	48	27	6	9
.. ..	199	43	..	6	..
R. D. ..	181	69	..	6	..
.. ..	180	76	..	6	..
.. ..	182	98	..	3	..

DISCUSSION.

From Fig. 1 which shows the average weekly increase in weight during 12 weeks, it is evident that the 'balanced diets', both original and modified, fall far below the standard for normal growth in rats as shown by that attained on the 'ideal diet'. They seem to be almost the same in their effect on growth as the 'routine diet' of the D. S. I. School. The averages for all 12 weeks for the three diets are as follows:—

Diet.	Average weekly increase in weight during 12 weeks in grammes.
1. Physiologically ideal diet (P. I. D.).	12.08
2. Balanced diet (B. D.)	7.13
3. Routine diet (R. D.)	5.70

Fig. 2 shows that the maximum response in growth to B. D. and R. D. is markedly lower than the response obtained with P. I. D. Here also the proximity of the growth curves on the former two diets is noticeable. Here reference must be made to the fact already mentioned that B. D. was modified early in April. The rats designed for feeding with B. D. were put on the experimental diet in three batches, on the 31st of January, 28th of February, and 13th of March, 1936. Thus, the first batch received the modified B. D. for the last four weeks, the second batch for the last six weeks and the third batch for the last eight weeks of the experimental period. Since the modifications were made to make the diets more nutritious, the change ought to have been reflected in the weekly increase in weights. That it is not so is amply shown by the graph for B. D. in Fig. 1.

The analytical figures in Table II show that the original B. D. and R. D. are poor in good quality fats and proteins, i.e., fats and proteins of animal origin. Inclusion of these in the diet in certain proportions is necessary as the former serve as a vehicle for the fat-soluble vitamins, while the latter effect economy of protein assimilation by providing the right type of proteins. An increase of animal protein from 29.7 to 36.9 per cent and of animal fat from 30.8 to 40.2 per cent in B. D. was not sufficient to produce any increase in the growth of rats. A quantitative assay of the essential amino acids in these diets (Table III) shows that with the exception of lysine, in which the original B. D. and R. D. are definitely poor, their distribution seems to be more or less even. Since information about the minimum intake of those amino acids required for growth or maintenance in human beings is not available, it is not possible to base any significant conclusion on the data in Table III beyond a mention that the lysine content might probably have been a limiting factor in determining the growth of rats.

During the whole of the experimental period the rats on P. I. D. showed no loss of hair and were generally healthy. Those on B. D. and R. D. lost a good deal of hair during the third and fourth weeks of feeding. Moreover, there were two deaths in the B. D. group, the causes of which could not be ascertained as no post-mortem examination was done.

It has not been possible to derive much information from the quantitative analyses of the diets for elements like iron, calcium, phosphorus, sulphur, and chlorine. All these seem to have been supplied in quantities above the minimum requirements postulated by Sherman (1923). From a scrutiny of Tables I, II, and III it appears that the better response to P. I. D. could possibly be ascribed to the additive influence of the following factors: (1) the high animal fat and protein content, (2) the inclusion of fresh whole milk, and (3) the high lysine content.

Since the 'balanced diet' is designed for consumption by adults it was thought desirable to follow up the growth of rats with observations on the effect of these three diets on their reproductive ability. Results given in Table IV show that the number of days which elapsed between the pairing of male and female rats and the arrival of the first litter varied according to the diet with which the rats were fed. The average number of such days for rats on P. I. D. was 24.8, for those on B. D. it was 36.4 and for the rats of the R. D. group the average was 81 days; the average number of young rats per litter in each group was 6.8, 5.6, and 5.0, respectively. The extreme slowness of response in case of the last diet may probably have been due to a variety of causes among which may be included (a) late maturity, (b) unsuccessful mating, or (c) resorption of the fertilized ovum. For want of further study no light can be thrown on this subject. Observations of this kind might, however, bring to light any effects which diets, planned or otherwise, may exert on the factors responsible for sexual reproduction.

SUMMARY.

The non-vegetarian 'balanced diets' advocated by the Bombay Presidency Baby and Health Week Association, a 'physiologically ideal diet' evolved in this Laboratory, and the 'routine diet' of the David Sassoon Industrial School for boys, Matunga, Bombay, have been examined analytically and also by observing their effect on the growth and reproduction of albino rats. On the basis of the experiments reported in the preceding pages, the following conclusions can be drawn:—

1. The 'balanced' and the 'routine' diets are relatively poor in fats and proteins of animal origin. The same diets are very poor in lysine which is an essential amino acid.
2. The average weekly increase in weights of rats on the 'balanced' and 'routine' diets was 7.13 g. and 5.7 g., respectively, as compared with 12.08 g. on the 'physiologically ideal' diet. A 10 per cent increase of animal fats and a 7 per cent increase of animal proteins in the original B. D. due to the addition of mutton fat and skim-milk powder, did not cause any rise in the average weekly increase in weights.

3. The reproductive power has been impaired only slightly in rats on the 'balanced' diet, but more markedly in rats on the 'routine' diet. Rats fed on the 'physiologically ideal' diet procreated according to expectations.

Further work on testing other cheap diets is in progress.

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THE ASH, CALCIUM, AND PHOSPHORUS CONTENT OF SOME COMMON BENGALI FOODSTUFFS.

BY

H. ELLIS C. WILSON, M.B., CH.B., D.SC.,

Professor of Biochemistry and Nutrition,

BASHIR AHMAD, PH.D.,

Assistant Professor of Biochemistry and Nutrition,

AND

D. N. MULLICK, M.Sc.

*(From the Department of Biochemistry and Nutrition ; All-India Institute of
Hygiene and Public Health, Calcutta.)*

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THE significance of minerals in nutrition needs no stressing, and an opinion on the intake of those substances can only be made when an analysis of the common varieties of foodstuffs is available. Furthermore, modern investigation on dietary habits has shown that all over the world the phosphorus and above all the calcium intake is often below what can be absorbed by the healthy growing child. The figures for ash, calcium, and phosphorus given in this paper are merely representatives of some of the common varieties of foods eaten in Bengal and purchased in the Calcutta market. Apart from their use in practical dietetics in this province a comparison with similar foodstuffs in other areas in India may not be without value when taken in conjunction with the local dietaries and/or any signs of deficiency disease. The figures given in the Table on the whole need no particular comment except to emphasize one or two points which should be noted by those concerned in food planning on a moderate scale of means. Atta and the dals are in general richer in calcium and phosphorus than rice. The vegetables as a whole are not particularly good sources of calcium except perhaps cabbage and in a lesser degree bhindi and spinach. This, however, need not detract from their use as they contain other important minerals apart from the accessory food factors. Above all, however, milk and milk products such as curds, sandesh, etc., are by far the best sources of

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calcium and in addition as shown by Sherman (1932) calcium in the form of milk is much better absorbed than in a food such as spinach.

TABLE.

Analysis of foods in g. per 100 g. material.

Foodstuff.	Total ash (g.).	Total Ca (g.).	Total P (g.).
ANIMAL FOODS.—			
Mutton	0·96	0·019	0·242
Fish (<i>hilsa</i>)	0·96	0·102	0·352
„ (<i>rôhita</i>)	1·30	0·073	0·314
„ (<i>tangra</i>)	0·60	0·065	0·302
„ (<i>kôî</i>)	1·30	0·131	0·252
Egg, hen	0·96	0·045	0·180
MILK AND MILK PRODUCTS.—			
Milk, cow's	0·642	0·120	0·092
Curd	0·680	0·138	0·112
Sandesh	1·70	0·590	0·360
Butter	Trace	Trace	Trace
Ghee	„	„	„
CEREALS AND PULSES.—			
Wheat (atta)	1·60	0·097	0·310
Rice	0·75	0·040	0·083
Suji (semolina)	0·56	0·060	0·150
Gram, whole	2·70	0·190	0·320
„ dal	2·30	0·106	0·406
Arhar „	3·20	0·059	0·476
Mung „	3·60	0·141	0·592
Beans	0·75	0·060	0·110
Lentils (masur)	1·60	0·064	0·384

TABLE—*contd.*

Foodstuff.			Total ash (g.).	Total Ca (g.).	Total P (g.).
NUTS.—					
Almonds	..	..	2.16	0.072	0.401
Coco-nut	..	..	1.32	0.034	0.191
VEGETABLES.—					
Potato	..	..	0.93	0.007	0.060
Carrots	..	..	0.99	0.058	0.055
Radish	..	..	0.78	0.035	0.025
Cabbage	..	..	0.74	0.134	0.039
Spinach	..	..	1.50	0.070	0.080
Tomato	..	..	0.54	0.011	0.032
Cucumber	..	..	0.50	0.023	0.049
Pumpkin	..	..	0.23	0.029	0.020
Brinjal	..	..	0.42	0.028	0.035
Bhindi (lady's fingers)	..	..	0.96	0.100	0.105
Patòl	..	..	0.44	0.030	0.100
Karela (bitter gourd)	..	..	0.842	0.035	0.048
Vegetable marrow	..	..	0.41	0.020	0.010
Jhinga (tôri) (ridge gourd)	..	..	0.50	0.010	0.020

TABLE—concl'd.

Foodstuff.	Total ash (g.).	Total Ca (g.).	Total P (g.).
<i>VEGETABLES—concl'd.</i>			
Lau (gourd)	0.72	0.030	0.009
Puin sak	1.08	0.070	0.096
Onion	0.58	0.026	0.062
<i>FRUITS.—</i>			
Banana	0.68	0.043	0.041
Grapes	0.56	0.075	0.025
Oranges	0.32	0.068	0.026
Papaya	0.48	0.054	0.026
Mango pulp	0.65	0.021	0.019
Jack-fruit	0.71	0.027	0.065

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THE CAROTENE CONTENT OF SOME COMMON BENGALI FOODSTUFFS.

BY

BASHIR AHMAD, M.Sc., PH.D.,

Assistant Professor of Biochemistry and Nutrition,

D. N. MULLICK, M.Sc.,

AND

B. N. MAZUMDAR, M.Sc.

*(From the Department of Biochemistry and Nutrition ; All-India Institute of
Hygiene and Public Health, Calcutta.)*

[Received for publication, July 29, 1936.]

THE importance of carotene as a source of vitamin A in human nutrition in India should be appreciated by all those concerned in practical nutrition. Foods rich in preformed vitamin A, such as milk, fish-liver oils, eggs, etc., are in general not available for most of the population and indeed among the poorer classes, carotene in the form of vegetables is their only protection against deficiency disease associated with this vitamin. The necessity for figures giving the carotene content of local foods is hence obvious. The data recorded here were obtained from analyses of foods bought in the local Calcutta market and comprise the cheapest and most widely consumed products used by the people.

METHOD OF CAROTENE ESTIMATION.

Five to ten grammes of material were ground up with pure sand and repeatedly extracted first with acetone and then with ether until no more of the pigments were removed and the extract was colourless. The combined extracts were transformed into a separating funnel, some more ether added and repeatedly washed with water to remove all the acetone. The ethereal solution was then saponified by shaking with 20 per cent alcoholic potash. It was then washed with water to remove the chlorophyll. If this solution still contained some green pigment, the saponification was repeated. The yellow ethereal extract containing only carotinoid pigments

was distilled under conditions preventing oxidation of the pigment (either by adding a trace of hydroquinone or in an atmosphere of an inert gas) until nearly the whole of the solvent was distilled off. The carotinoid pigments were then taken up in petroleum ether and repeatedly washed with 60 to 70 per cent alcohol to separate xanthophyll, etc. The petroleum ether extract was then washed once or twice with distilled water to remove any trace of alcohol, dried over anhydrous sodium sulphate, brought to a known volume and the carotene estimated colorimetrically. A Lovibond tintometer was used for the estimation. A standard curve of carotene units was first prepared from different known concentrations of pure carotene and all values were derived by reference to this curve.

In this paper (see Table) it is sufficient to point out foods which are rich in this constituent. Among three classes of foodstuffs, namely, vegetables, fruits, and cereals, only the former can be considered as effective sources of this material.

It will be seen that vegetables of the leafy type, particularly *saks*, are the richest; *soya sak*, for instance, contains some 10 mg. per 100 g. The tuberous vegetables on the other hand are relatively carotene-poor, except carrots. Radish leaves, for instance, contain about 8 mg., while the radish itself has almost no carotene. With the exception of the mango, the common fruits and cereals are for all practical purposes carotene-free.

As far as the practical application of the results is concerned, it should be pointed out that there is good reason to believe that carotene can replace the preformed vitamin in most respects, possibly all, but this is not yet an established fact. Further, the relative absorbability of carotene under different conditions and its transformation into the vitamin itself are both factors which must be taken into consideration in assessing its value. This, however, should in no way prevent its increased use, particularly as such substances as the *saks* are popular and easily grown in the plains or hills provided the minimum of moisture is present.

TABLE.

The carotene content of some Bengali foods.

Number.	Foodstuff.	Mg. carotene per 100 g. of material.
VEGETABLES.—		
1	Spinach	5.6-6.5
2	Pumpkin leaves ..	5.75-7.2
3	Polla sak	6.2-6.5
4	Soya sak	10.8-11.6
5	Lal sak	6.75
6	Meti sak	4.0-4.5
7	Puin sak	3.2-3.5

TABLE—*contd.*

Number.	Foodstuff.	Mg. carotene per 100 g. of material.
VEGETABLES— <i>contd.</i>		
8	Radish leaves	7·2-8·6
9	Lettuce	1·5-1·94
10	Mint (<i>podina</i>)	3·3-4·6
11	Cauliflower leaves	3·3-3·9
12	<i>Kalmi sak</i> (<i>ipomœa</i>)	5·2-5·5
13	Radish	Traces
14	Potato	"
15	Brinjal	"
16	Turnip	"
17	White turnip	<i>Nil</i>
18	Pumpkin	Traces
19	Garlic	"
20	Onion	"
21	Green banana pulp	<i>Nil</i>
22	<i>Rangalu</i> (sweet potato)	0
23	Squash	Traces
24	French beans	0·35-0·4
25	<i>Lau</i> (gourd)	0·16
26	Cucumber	0·175-0·2
27	Beet root	0
28	Cauliflower	Trace
29	Green mango (without skin)	"
30	Country peas (green)	1·94-2·0
31	Red pepper (green)	0·75-0·9
32	Carrot	2·0-5·6
33	<i>Kachu</i> (<i>arum</i>)	0

TABLE—concl'd.

Number.	Foodstuff.	Mg. carotene per 100 g. of material.
<i>ANIMAL FOODS—concl'd.</i>		
79	<i>Kôî</i> fish—flesh ..	Trace
	„ „ liver ..	„
80	<i>Rôhita</i> fish—flesh ..	0·06
	„ „ liver ..	1·22
81	Egg-white ..	0
82	Egg-yolk—hen ..	0·14
	„ duck ..	0·134

STUDIES ON THE ABSORPTION OF CAROTENE AND VITAMIN A IN THE HUMAN SUBJECT.

BY

H. ELLIS C. WILSON, M.B., CH.B., D.SC.,

*Professor of Biochemistry and Nutrition ; All-India Institute of Hygiene
and Public Health, Calcutta,*

S. M. DAS GUPTA, M.SC.,

Chemistry Department, Medical College, Calcutta,

AND

BASHIR AHMAD, PH.D.,

*Assistant Professor of Biochemistry and Nutrition ; All-India Institute of
Hygiene and Public Health, Calcutta.*

[Received for publication, July 29, 1936.]

THE metabolic rôle of carotene and its efficacy as a pro-vitamin A in curing certain symptoms associated with vitamin-A deficiency in rats has been conclusively proved for some time. That it does play a similar part in human nutrition has special significance in India where carotene is the only source of this vitamin for most of the people. The necessity for investigating in the human subject, some at least of the factors which are concerned in its absorption, is hence of considerable importance.

While doing this experiment we also took the opportunity to follow the absorption of a commercial concentrate of vitamin A. This was of special interest in view of the fact that the vitamin was either free of its natural solvent or in an abnormal concentration in some fatty medium. Further, some clinicians have informed us that in many cases they have the impression that cod-liver oil as a therapeutic agent is more effective than the special preparation. The main object, however, of this investigation was to determine how far carotene in the form of two kinds of vegetables rich in this substance, namely, carrots and spinach, was absorbed on a typical Indian diet with and without fat. One of us (S. M. D. G.), a healthy Bengali male of 29 years of age and 147 lb. body-weight, acted as the experimental subject.

The general technique employed was that of the superimposition method which has been used for many years in metabolic balance experiments. A standard carotene-free diet was consumed during the whole course of the experiment which was divided into three periods; period I, standard diet alone (usually 4 days); period II, standard diet plus the food to be investigated (4 days); and period III, standard diet again (4 days). Another food could then be taken up making a fourth period if necessary. The faeces were collected in two-day periods and the excretion of carotene calculated therefrom. The excretion of carotene obtained in the basal period was then subtracted from the values for the experimental period in order to obtain the true figure for the unabsorbed carotene when the specific foodstuff was being ingested. The basal diet consumed was as follows:—

	Oz.
1. Rice (polished)	16
2. Pulses: (lentils, <i>mung</i> , <i>arhar</i>)	2
3. Fish	4
4. Vegetables: (potato, <i>patôl</i> , white turnip, gourd, pumpkin).	4
5. Mustard oil	$\frac{1}{2}$
6. <i>Ghee</i>	1
7. Condiments, small quantities	

In experiments III, IV, and V the fat (*ghee* and mustard oil) was omitted. The carrots were ingested raw, while the spinach was cooked in water, which was afterwards consumed. The results are summarized in the Table. The carotene in the raw materials (carrot and spinach) was estimated as follows:—

TABLE.

Period.	Length of period (days).	Total amount of foodstuff ingested (g.).	Total amount of carotene ingested (mg.).	Total amount of carotene excreted (mg.).	Carr-Price reaction of the fecal extract.	Net total carotene absorbed (mg.).	Percentage carotene absorbed.
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Experiment I.—CARROTS (ordinary basal diet).

I	(a)	3	0	0	2.377	Negative	..	..
	(b)	2	0	0	0.631	Negative	..	..
II	(a)	3	170	16.40	4.10	Positive	} 21.28	77.83
	(b)	2	113	10.94	3.544	Faint		
III	(a)	2	0	0	2.08	Faint	..	..
	(b)	2	0	0	0.68	Negative	..	..
IV	(a)	2	320	30.91	4.724	} Strongly positive	} 53.78	86.99
	(b)	2	320	30.91	4.680			

TABLE—concl'd.

Period.	Length of period (days).	Total amount of foodstuff ingested (g.).	Total amount of carotene ingested (mg.).	Total amount of carotene excreted (mg.).	Carr-Price reaction of the faecal extract.	Net total carotene absorbed (mg.).	Percentage carotene absorbed.
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Experiment II.—SPINACH (ordinary basal diet).

I	(a)	2	0	0	0.454	Negative	..	..
	(b)	2	0	0	0.405	Negative	..	..
II	(a)	2	113	5.244	1.125	} Faint	} 9.319	88.85
	(b)	2	113	5.244	0.954			
III	(a)	2	0	0	0.781	Faint	..	..
	(b)	2	0	0	0.402	Negative	..	..

Experiment III.—SPINACH (fat-free basal diet).

I	(a)	2	0	0	0.62	Faint	..	..
	(b)	2	0	0	0.56	Very faint	..	..
II	(a)	2	113	5.244	2.96	} Strongly positive	} 5.488	52.32
	(b)	2	113	5.244	3.22			
III	(a)	2	0	0	0.81	Faint	..	..
	(b)	2	0	0	0.66	Very faint	..	..

Experiment IV.—VITAMIN-A CONCENTRATE (ordinary basal diet).

I	..	4	0	0	..	Negative	..	..
II	..	2	2.0	..	..	*Negative	..	..
III	..	2	4.0	..	..	*Negative	..	..

Experiment V.—VITAMIN-A CONCENTRATE (fat-free basal diet).

I	..	2	0	0	..	Negative	..	..
II	..	2	4.0	..	..	*Negative	..	..

* In these cases a pink coloration was obtained with SbCl_3 reagent.

The raw material was cut into slices or small pieces and freed from extraneous matter, etc. About two grammes of it was macerated with about half a gramme of sand and then successively extracted with ethyl alcohol, acetone and ether until no yellow coloration was obtained with the latter which is the main solvent. The ethereal layer was then washed free from alcohol, acetone, etc., and then saponified in the cold with 20 per cent ethyl-alcoholic potash for some 15 minutes in a separating funnel. The potash was washed off with water and the final ethereal solution was dried with fused sodium sulphate and evaporated to dryness. The residue was taken up with petroleum ether, washed with 60 per cent ethyl alcohol, dried with fused sodium sulphate and the solution made up to a known volume. The carotene was estimated calorimetrically by the Lovibond tintometer. By this method it was found that raw carrots contain about 9.66 mg. per hundred grammes and raw spinach about 4.6 mg. per cent of carotene.

In the case of the faeces, about 2 grammes to 3 grammes of it was macerated with an excess of fused sodium sulphate in a mortar and then the carotene extracted with ether. The procedure was then similar to that described above for the vegetables.

DISCUSSION.

It will be seen from the Table that the faeces extract gave a figure in yellow units although the Carr-Price reaction was negative. The nature of this substance, which, however, was present only in traces, is unknown. The figure obtained, however, expressed in yellow units, was subtracted from the values obtained during the experimental period when the reaction became positive showing that there was some excretion of carotene.

As regards the absorption of carotene, it will be noted—experiments I and II—that the retention is equally good with either carrots or spinach. The chemical state in which the substance is present in the vegetables is apparently favourable for an almost complete absorption provided fat is present in the diet. An increase in the quantity of carrots in the second part (period IV—experiment I) apparently has, at that level of intake, no adverse effect on the absorption, 15 mg. per day being absorbed as efficiently as 5 mg. or 6 mg. The effect of the presence or absence of fat in the diet on the absorption of carotene from spinach is clearly shown in experiments II and III. In experiment II with fat, out of 10.49 mg. ingested, 9.318 mg. were absorbed, while in experiment III, under identical conditions except for the absence of fat, only 5.488 were retained.

This observation may be of considerable importance in India where all over there tends to be a scarcity of fat and in certain areas where vegetables are scarce, this may be the decisive factor in determining the appearance of signs of deficiency disease.

Absorption of vitamin A.—Experiments IV and V give the results obtained when a commercial preparation of vitamin A was taken on a fat-free diet. The equivalent of 15,000 to 30,000 International Units of vitamin A were ingested in those experiments. The antimony trichloride test was done on an extract of faeces similar to that employed for the carotene estimation. In both experiments the test was negative. One can hence conclude that apart from bacterial decomposition in the intestine, these results favour the assumption that all the vitamin has been absorbed.

The absorption of the isomers of carotene.—The excretion of carotene in fairly large quantities in experiment III led the authors to examine in a preliminary way the nature of the carotene excreted. The total carotene was extracted from 785 g. faeces of period II—experiment III and the angle of rotation observed. The total carotene recovered was 6.18 mg. and the angle of rotation was 0.25° . The optical rotation of the *alpha* form is $+326^\circ$ and that of *beta* nil (Strain, 1935). The amount of the *alpha* form in the solution is hence given by the equation :—

$$X = \frac{15 \times 0.25}{2 \times 326} = 5.75 \text{ mg.}$$

The percentage of *alpha* carotene excreted is hence 93.04, while that of the spinach ingested was only 24.4. Apparently on the fat-free diet the active form *beta* carotene has been almost completely and selectively absorbed. The validity of this observation is dependent on the absence in the faecal extract of dextro-rotatory substances other than *alpha* carotene. Further work is in progress on this point which is of considerable interest in view of the fact that the two isomers in their chemical reactions are almost identical.

CONCLUSIONS.

Carotene ingested by the human subject on a typical Bengali diet appears to be absorbed equally well, either when fed raw as carrots or cooked in the form of *sak* (spinach).

The percentage of absorption of carotene on a fat diet is about 90 in contrast to about 50 on the similar diet without fat. There is evidence to show that the body may preferentially absorb the physiologically active *beta* isomer of carotene.

There is evidence that a highly concentrated extract of vitamin A is, apart from bacterial decomposition, completely absorbed.

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THE VITAMIN-B₁ CONTENT OF SOME COMMON INDIAN FOODSTUFFS.

BY

H. ELLIS C. WILSON, M.B., CH.B., D.SC.,

Professor of Biochemistry and Nutrition,

BASHIR AHMAD, M.SC., PH.D.,

Assistant Professor of Biochemistry and Nutrition,

G. RAY, M.SC.,

AND

R. C. GUHA, M.SC.

(From the Department of Biochemistry and Nutrition; All-India Institute of Hygiene and Public Health, Calcutta.)

[Received for publication, August 18, 1936.]

THE investigation recorded below covers the analysis of some 40 representative samples of common Indian foods for their vitamin-B₁ content. The number analysed is unfortunately not very large owing to the time involved in biological assays of this type. The data recorded, however, should be for the most part adequate to give an opinion on the vitamin-B₁ intake of the average Indian and above all Bengali consuming his accustomed diet. The method employed is that of the rat growth technique elaborated by Roscoe (1930). Young rats of 30 g. to 45 g. weight were put on the following B₁-free diet:—

	Per cent.
Casein	15
Dextrine	65.5
Yeast autoclaved	10.0
Butter fat	2.5
Salt	5
Cod-liver oil	2

When the body-weight curve began to fall, a weighed quantity of the food to be investigated was given, care being taken to see that it was all consumed. In the case of the vegetables a small weighed piece of the sample was given the animal each day, while with the cereals a certain percentage of the dextrine was replaced by the cereals to be tested and the total food consumed each day obtained by weighing the special (Slonaker) food pan. The intake of cereal which produced the requisite growth could then be calculated.

The unit of B₁ was taken as that quantity which when given each day would produce an increase of weight of 10 g. per week for at least three weeks. Most of the foods were tested on at least 6 rats in order to find the quantity which would give as exactly as possible a 10 g. increase. In some cases where the average increase was a little more or less than 10 g. per week a calculation of the vitamin units was made from a graph of Coward *et al.* (1933). This method in most cases appeared to give results which agreed with the figures obtained when a growth of exactly 10 g. per week was obtained. The results are all expressed as units of vitamin B₁ per 100 g. food. This unit (Roscoe) is equal to an International Unit which is equivalent to 4 micrograms of the Jansen and Donath crystals. The investigation covered vegetables and cereals and a glance at figures (*see* Table) will show that the vegetables are all relatively poor in B₁, the number of units varying from 11 to 40 per 100 g. The cereals, however, are all comparatively rich in this accessory foodstuff and particular attention should be drawn to green *mung*, *arhar* dal, lentils and beans. The mean value for all cereals is round about 150 with the exception of polished rice which has only some 26 units per 100 g. in contrast to atta with 110. As rice or atta form the staple background of diets in India the significance of the results needs no stressing. The superiority of the dals is off-set to a certain extent by the fact that they are not and cannot be consumed in such quantities as rice or atta.

TABLE.

Number.	Foodstuff.	Units of vitamin B ₁ per 100 g. of material.
VEGETABLES.—		
1	<i>Patól</i>	16-20
2	Brinjal	19-20
3	<i>Karela</i> (bitter gourd) ..	22-25
4	Tomato	22-24
5	<i>Jhinga</i> (ridge gourd) ..	20-23
6	<i>Lal sak</i>	10-13
7	Banana (green), without skin ..	12-17
8	Potato	20-30
9	<i>Kachu</i> (arum)	22-25
10	<i>Kumra</i> (pumpkin)	18-24
11	Peas (green)	20-25
12	Spinach	18-20
13	<i>Lau</i> (gourd)	19-20
14	Cabbage	20-25

TABLE—concl'd.

Number.	Foodstuff.	Units of vitamin B ₁ per 100 g. of material.
VEGETABLES—concl'd.		
15	Turnip	12-14
16	Cauliflower	15-20
17	Squash	17-18
18	Carrot	20-24
19	Cucumber	18-20
20	Bhindi (lady's fingers) ..	20-22
21	Rangalu (sweet potato) ..	11-13
22	Kalmi sak (ipomœa) ..	25-30
23	French beans	25-26
24	Puin sak	21-39
25	Papaya (green)	25-30
26	Radish	30
27	Onion	16
CEREALS.—		
28	Gram	90-96
29	Green mung	150-160
30	Black mung	140
31	Sona mung	115
32	Lentils	150
33	Maize	70
34	Arhar	190
35	Beans	150-170
36	Wheat (atta)	100-110
37	Millet (bajri)	110
38	Barley	150
39	Polished*rice	26
40	Peas	150

It is not intended to discuss the vitamin-B₁ requirements of man, as a paper based on diet survey on this subject is already projected and will be published later. It should be mentioned, however, that from the data given by Cowgill (1934) the amount of vitamin-B₁ units to protect from beri-beri is assessed at about 150 to 350. This, however, is a protective dose and as yet we have no knowledge as to the optimum requirements of man for this substance.

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NUTRITIONAL SURVEY OF SCHOOLBOYS IN CALCUTTA
AND THE PUNJAB BY MEANS OF CLINICAL
OBSERVATIONS, A. C. H. INDEX OF
NUTRITION AND OTHER
MEASUREMENTS.

BY

H. ELLIS C. WILSON, M.B., CH.B., D.Sc.,

Professor of Biochemistry and Nutrition,

BASHIR AHMAD, PH.D.,

Assistant Professor of Biochemistry and Nutrition,

AND

DURGA DAS MITRA, M.B., M.Sc., D.P.H.

(From the All-India Institute of Hygiene and Public Health, Calcutta.)

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THE criticism of diets as adequate or inadequate in the light of their chemical composition, useful though it may be, must ultimately be correlated with the health of the community subsisting on such diets. The investigation recorded in this paper is intended as an attempt to find standards of nutritional status and the incidence of malnutrition among the children in the areas concerned. The fields of study cover some 3,000 boys of varying economic classes and of different communities in Calcutta and 1,250 of more or less the same economic class but of different 'communities' in Ferozepore (Punjab). The original object of the investigation was to test out a special method for estimating defective development or malnutrition called the Arm-Chest-Hip index of nutrition (henceforth A. C. H. index). This method devised by Doctors Franzen and Palmer of the American Child Health Association consists essentially of the following :—

A. C. H. INDEX.

The original purpose of the measurements and the ratios obtained by this index was to select out children who should be subjected to further medical examination for defects or disease. The originators devised the technique from a selection of a number of different skeletal and muscular measurements, finally

adopting on the basis of their constancy and significance the three mentioned, namely: arm, chest, and hip. All measurements are made in centimetres. The arm girth is measured by the maximum girth of the biceps (*a*) when the arm is hanging down and (*b*) when the tip of the fingers are touching the shoulder, care being taken to avoid marked contraction (making) of the biceps. The two readings are then summed. The chest depth is measured antero-posteriorly at the nipple with a pair of sliding callipers. The readings at normal inspiration and expiration are taken and summed. The hip width is measured with the same callipers from the heads of the trochanters, the subject standing with the heels and toes together. The difference between the sum total arm girth and that of the chest depth is found, and from the tables it is noted that with a certain hip width this difference should be above a certain figure. If it is less than the standard figure in the table the child is selected for further medical attention. The particular set of figures employed depends on how rigid a selection of defectives is desired. It should be noted that the method does not select all who should be subjected to further medical examination, and vice versa others are taken who may be quite normal. The term *selected* has been employed by the originators to designate those who are below an arbitrary physical standard.

In general, it would appear that the essential aim of the method is to show whether the child has an adequate proportion of soft tissue to skeleton. In order to find out the applicability of the method in this country, the height and weight were taken and observations on certain signs of deficiency disease and other abnormalities were looked for. As far as Calcutta is concerned, the scope of the inquiry widened as the investigation proceeded. All the children were examined for (*a*) phrynoderma (toad-skin), noted by Nicholls (1934) in Ceylon and others elsewhere in the tropics; the condition appears to be a deficiency disease, the exact ætiology of which is unknown, (*b*) angular stomatitis, a similar condition with an unknown ætiology, and (*c*) Bitot's spots due to vitamin-A deficiency.

During the course of this survey it was noted that the incidence of enlarged tonsils and carious teeth appeared to be high, so data on these points were collected from then on. Recently, Nicholls (1936) has described certain conditions called malocclusion and displaced teeth which he has found to be correlated with economic status. This unfortunately up to the present has not been recorded. One of us (H. E. C. W.), however, was struck early in the investigation in Calcutta by the number of children with unsymmetrical or displaced teeth. Data are now being kept on this point. The tongue was always observed but nothing abnormal was noted. Few cases of obvious anæmia were seen. The data collected in the Punjab cover the three communities: Hindu, Mohammedan, and Sikh, all more or less of the lower middle class of shopkeepers, artisans, etc. Observations on the skin, eyes, teeth, etc., were made similarly to what had been done in Calcutta. The observer (B. A.) of this particular survey, however, does not wish to stress the eye or skin condition, as he has not had experience in those conditions. The absence of any marked positive case would appear to indicate, however, that they are not common.

CALCUTTA AREA RESULTS.

The most striking points to note about the children of the same community but of different economic class is the contrast between their heights and weights

(see Figs. 1 to 8 and Tables I to IX). With the exception of the Marwari community, the Hindus, Mohammedans, and Anglo-Indians all showed this difference. The Marwaris are a partial exception, as the poorer class are probably Hindustanis and not completely vegetarian. This fact may hence account to a certain degree for the greater average height of the poorer class. Both classes were, however, of about the same weight, a feature which may be partially accounted for by the fact that the poorer classes do manual labour. The fact remains that the poor Marwaris have less muscle tissue in proportion to height than the others.

Incidence of disease or other defects.

The incidence of conditions such as toad-skin and Bitot's spots is for all practical purposes *nil*. Only one questionable case of toad-skin was seen and no active corneal ulceration was recorded. The milder degrees of Bitot's spots are probably easily missed, but, if there had been any tendency to such conditions, it is to be expected that more advanced cases would have been observed. The two conditions which showed a marked correlation with economic status were the carious teeth and enlarged tonsils. The most marked instance is that of the Marwari community (see Tables VI and VII) where the figures for the incidence of both enlarged tonsils and caries together in one child are 23 per cent for the poor and only 8·3 per cent for the better-off class. It should be pointed out, however, that enlarged tonsils may not be by any means due directly to nutritional defects; local hygiene, overcrowding, etc., may all play a part. A similar correlation between economic status and those two conditions singly or combined is found in all the communities, the highest incidence being 56 per cent for caries among the poor Anglo-Indians. This condition is in all probability partially associated with a deficiency of calcium in the diet. In favour of this is the observation that the lowest figure of all is among the Marwaris (21 per cent), who consume milk and atta both of which are good sources of minerals. The high figure for the Anglo-Indians, 56 per cent, is probably associated with a low milk intake and the consumption of white bread and rice without atta. It is unlikely, however, that the calcium intake is the only factor concerned as no cases of rickets were noted.

A. C. H. index.

The American investigators chose standard figures for the index so that 10 per cent of the children would be selected as below par as compared with the others. In Calcutta, using those standards, differences in the percentages selected in the different groups examined have been found but no positive correlation between height, weight, or economic status on the one hand and the index figures on the other have been noted. The figures for the better-off Marwaris, Bengali Hindus, and Bengali Mohammedans are 4·1 per cent, 16·3 per cent, and 19·8 per cent, while the corresponding figures for the poor classes are 1·0 per cent, 1·5 per cent, and 2·8 per cent, respectively. No correlation of the index with the incidence of such conditions as caries, enlarged tonsils, or even underweight has been observed. As regards the Anglo-Indian community, the percentage selected in both economic classes is as low as 3·7. This seems the more remarkable in view of the fact that almost certainly the lower class children must have been of poorer physique

FIG. 1

CALCUTTA

British -----
 Rich Bengali Hindus. ---●---
 Poor " " ---○---

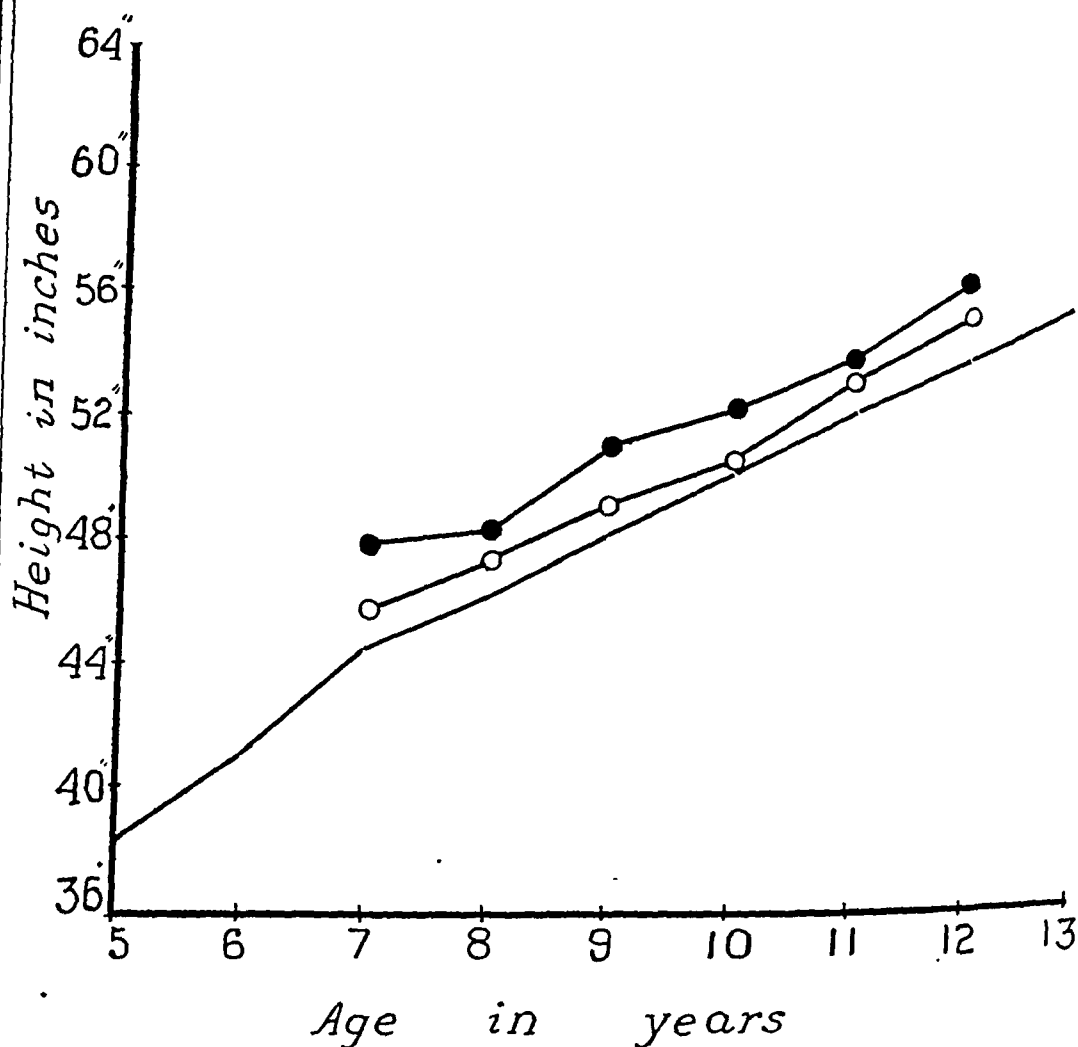
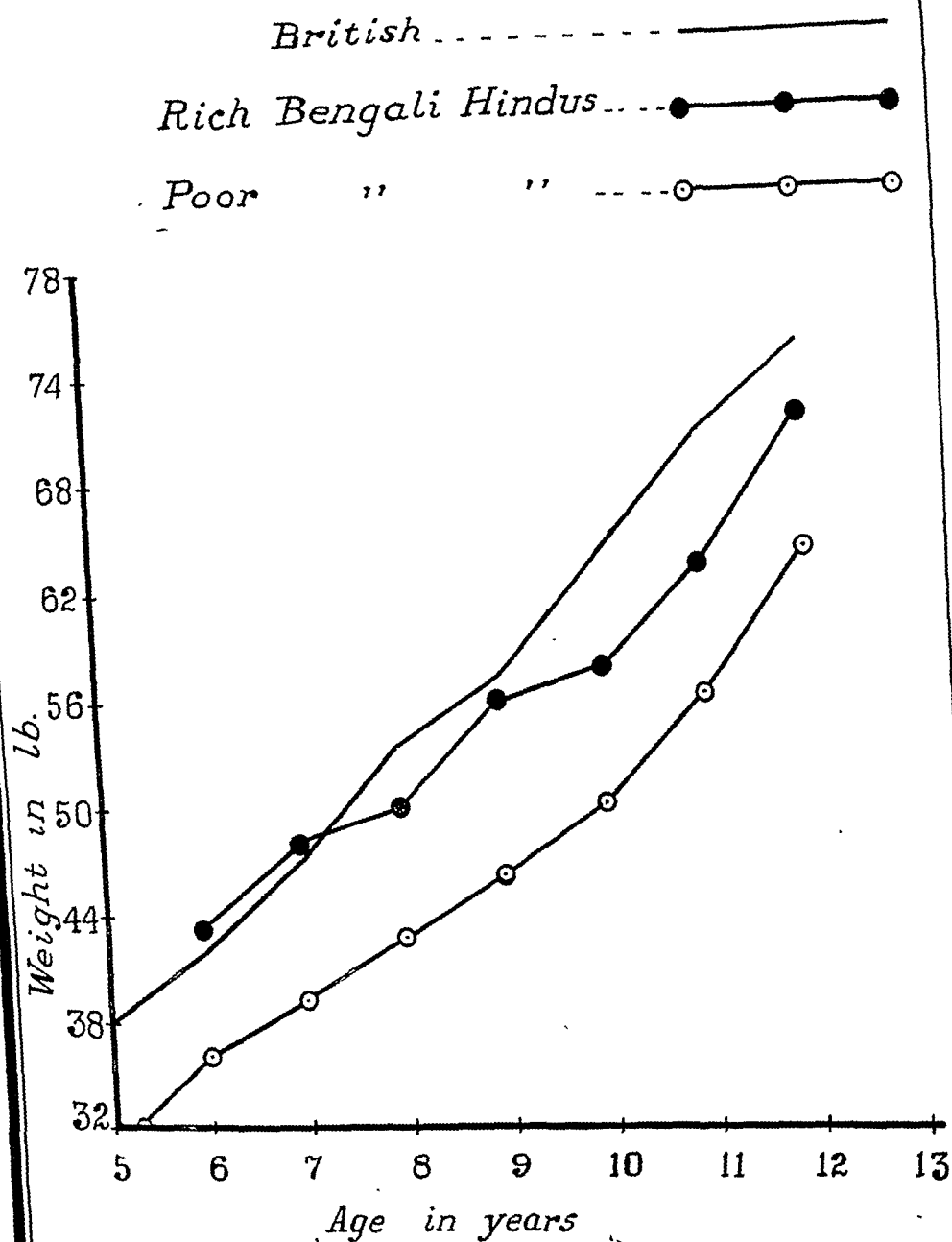


FIG. 2.

CALCUTTA



CALCUTTA

FIG. 4.

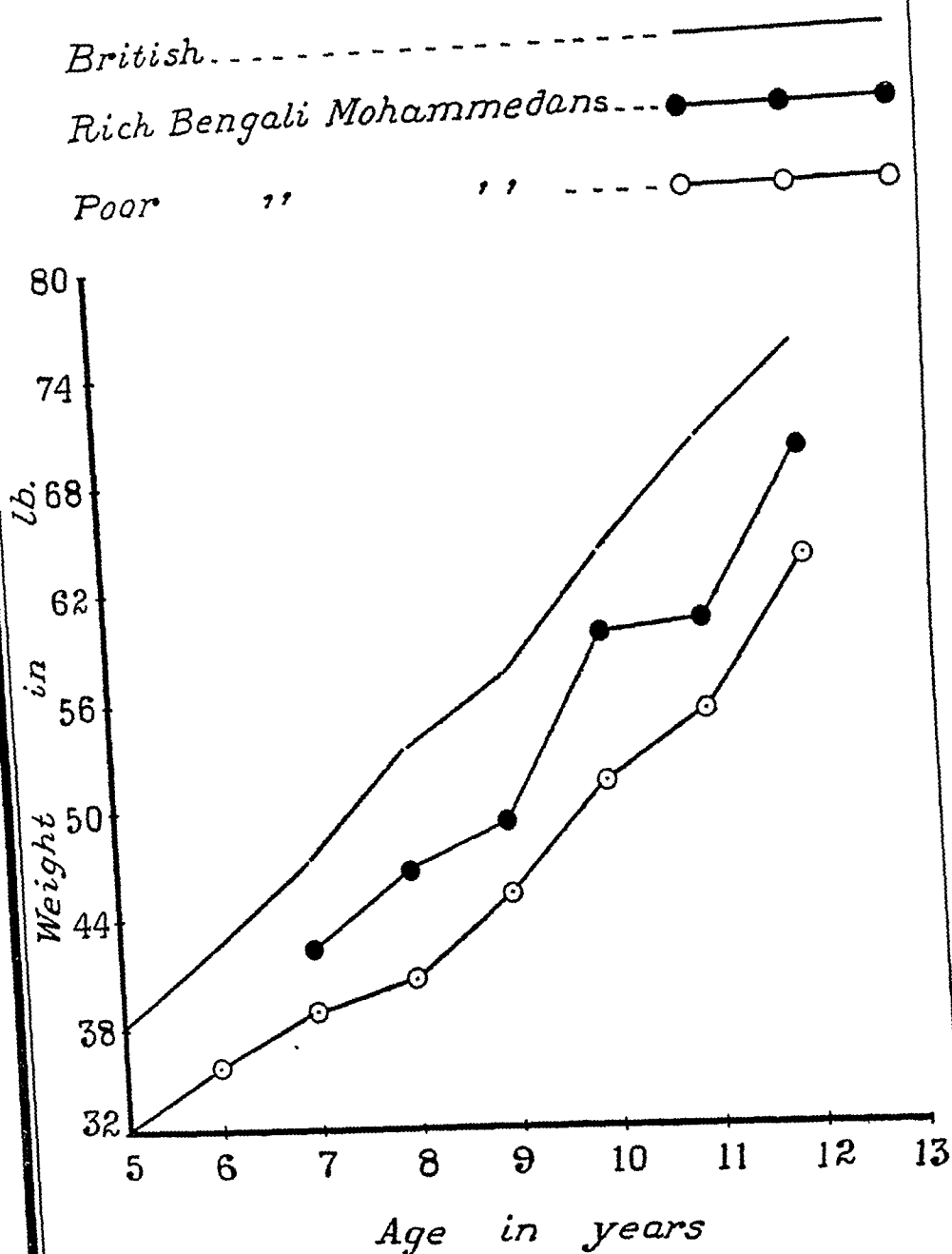


FIG. 5. CALCUTTA

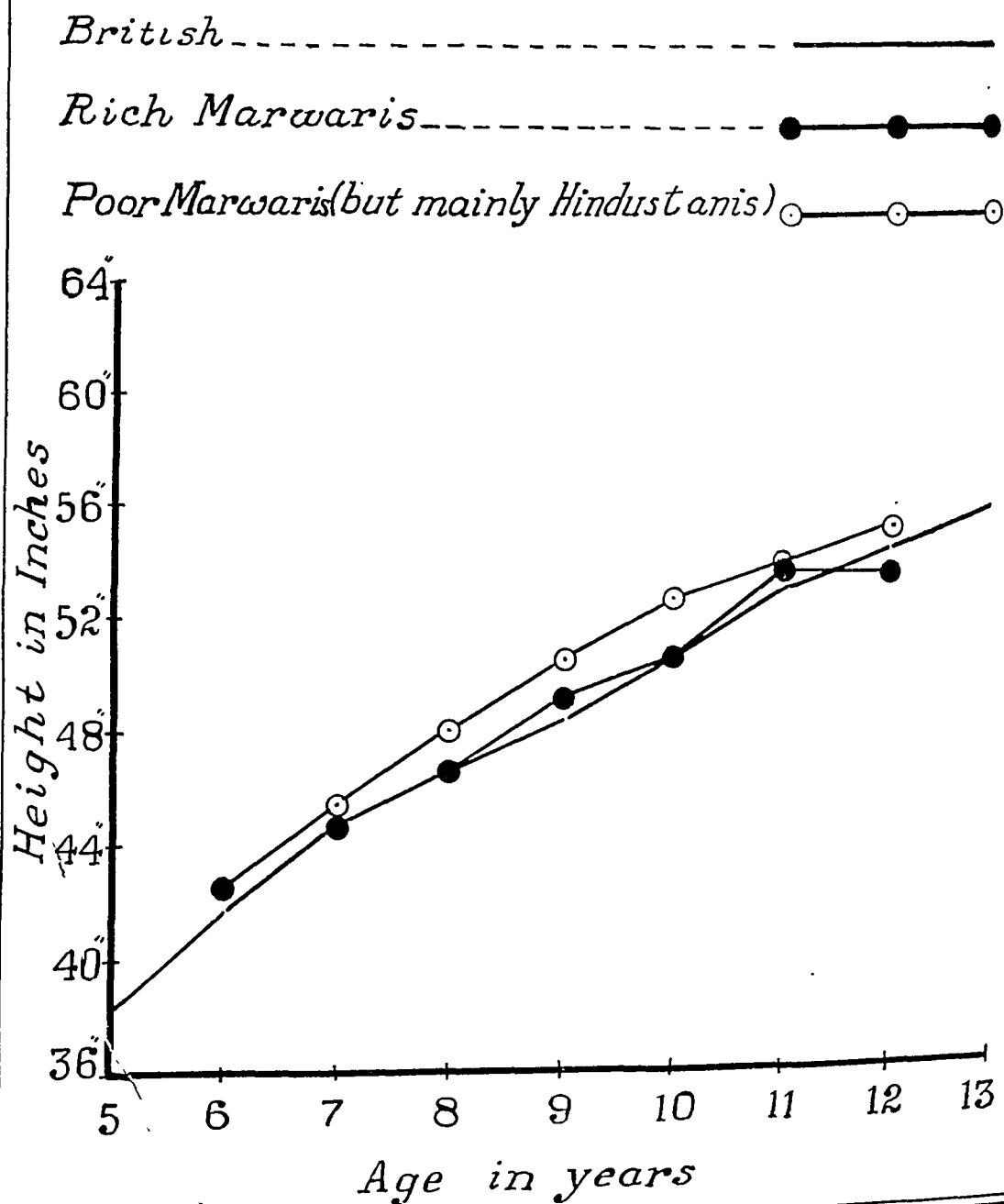


FIG. 6.

CALCUTTA

British -----
Rich Marwaris -----
Poor Marwaris (but mainly Hindustanis) ○-----

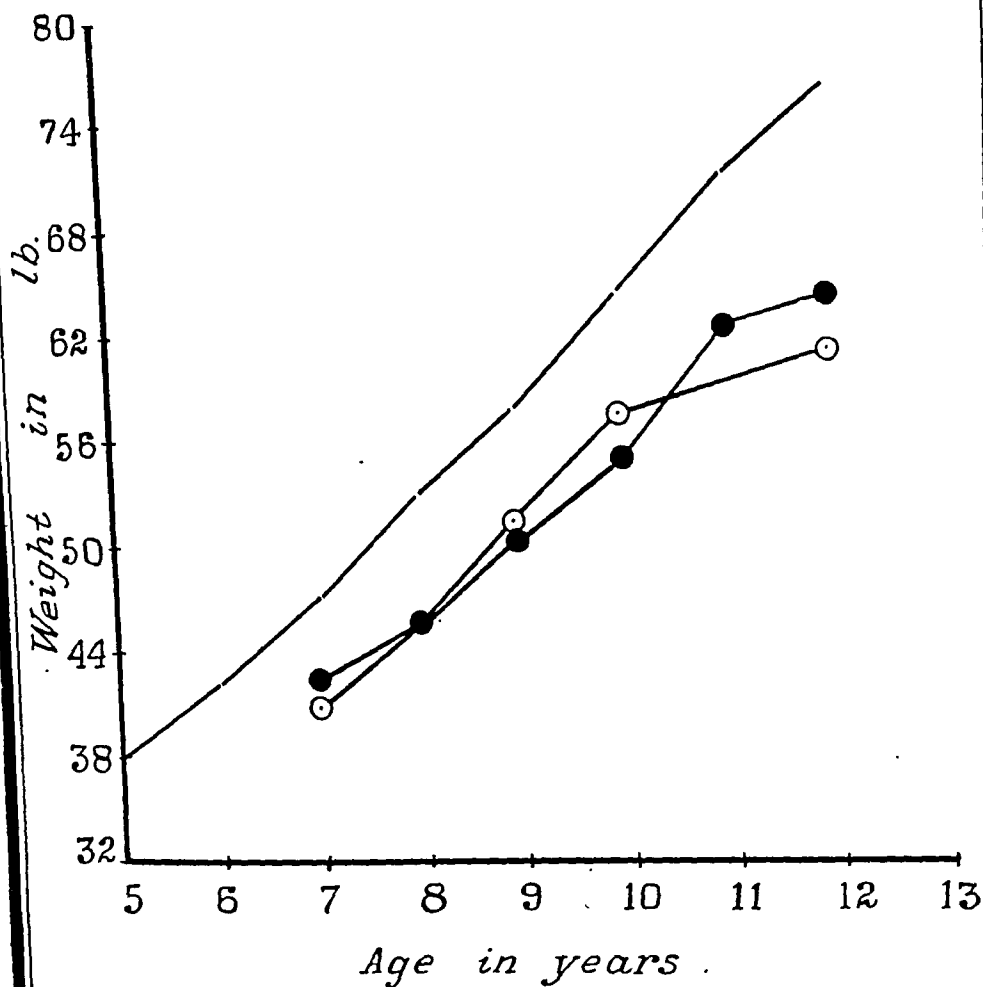
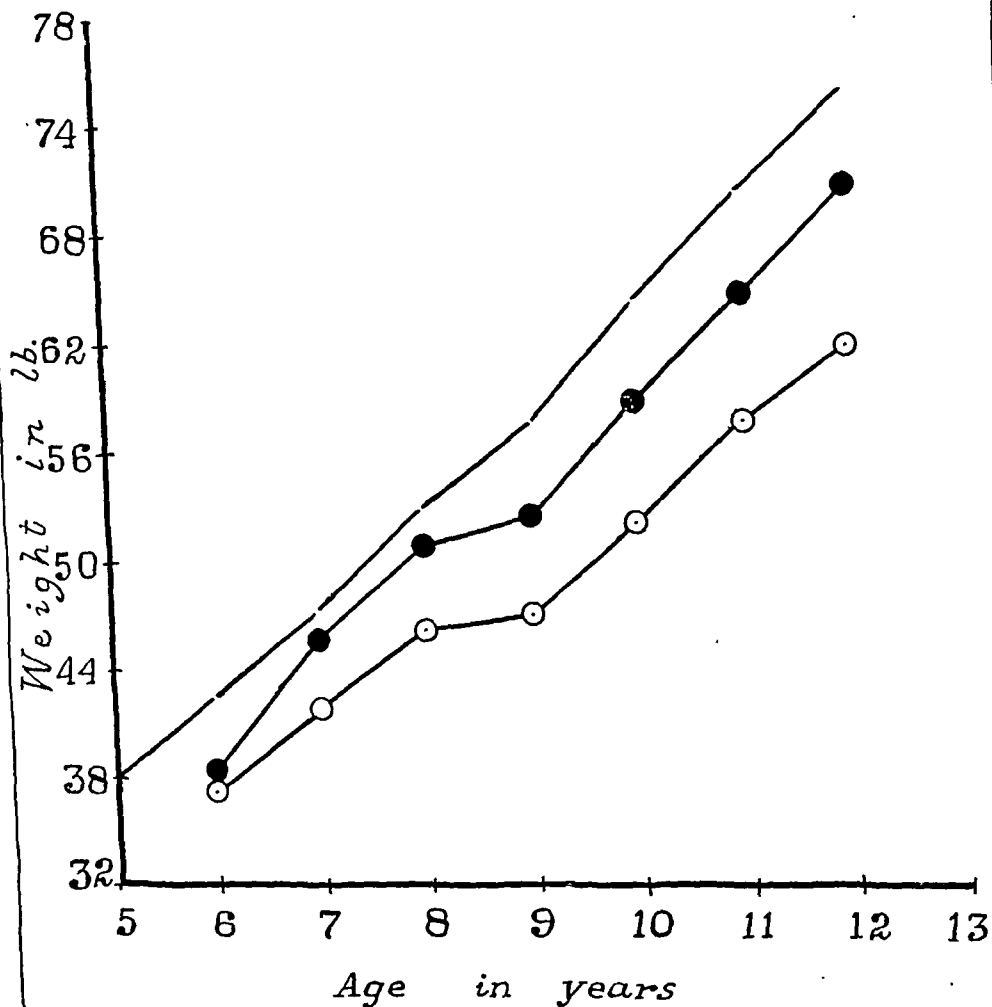


FIG. 8

CALCUTTA

British -----
Rich Anglo-Indians ----- ● ----- ● ----- ● -----
Poor " " ----- ○ ----- ○ ----- ○ -----



than their American brethren and yet *a priori* racially and hence physically should not differ from them so much as might the pure Indian. In other words, a selection of at least 10 per cent might have been expected among this community. More work will certainly have to be done on this subject particularly in regard to finding standards applicable to India itself.

PUNJAB INVESTIGATION RESULTS.

The 1,250 children of the three communities as mentioned above were all of the same economic class and in addition were in mixed schools. It would appear likely that the money available for food was approximately the same for all and that the difference in diet was probably not so marked as it might be elsewhere. All communities (B. A.) consumed flesh within the restrictions imposed by their beliefs and it would appear that a difference in diet was not very marked.

Before commencing the measurements, the children of each school were lined up and divided into four groups according to their physique, namely, good, fair, moderate, and poor. This arbitrary classification proved to be very significant.

Height and weight.

The heights and weights of the three communities do not show any particular difference beyond the racial one which is noted in the increase in rate of growth and increase of weight of the Sikhs at about 11 and 12 years of age (Figs. 9 and 10).

Incidence of disease.

The incidence of caries varies between 30 per cent and 38 per cent, the Mohammedans representing the smaller figure which is approximately the same as that found among the poor members of this community in Calcutta, viz., 27 per cent. The figures for enlarged tonsils are approximately the same for all communities ranging between 24 per cent and 27 per cent and approaches what is found among the better classes (excluding Anglo-Indian) in Calcutta. As this was a rural area without overcrowding and real urban conditions, the local hygienic environment may have accounted for this feature.

A. C. H. index.

The index in this particular area shows in contrast to Calcutta (see Table X) a definite positive correlation with the clinical assessment of physique. The percentages selection among the three communities, Hindu, Mohammedan, and Sikh, are 5.6, 10.1, and 6.9 respectively. If, however, only those children assessed as of poor physique are taken for analysis, the percentages selected are 28.7, 30, and 40, respectively, for the three communities. Further, if only those children who declared that they consumed milk regularly are subjected to analysis, again a positive correlation is noted. This difference is most marked in the case of the Mohammedans where the total selection is 10.1 per cent, while of those taking milk the corresponding figure is only 1.3 per cent.

Here as in Calcutta there is no consistent correlation between underweight and the incidence of caries or enlarged tonsils and the index.

PUNJAB

FIG. 9.

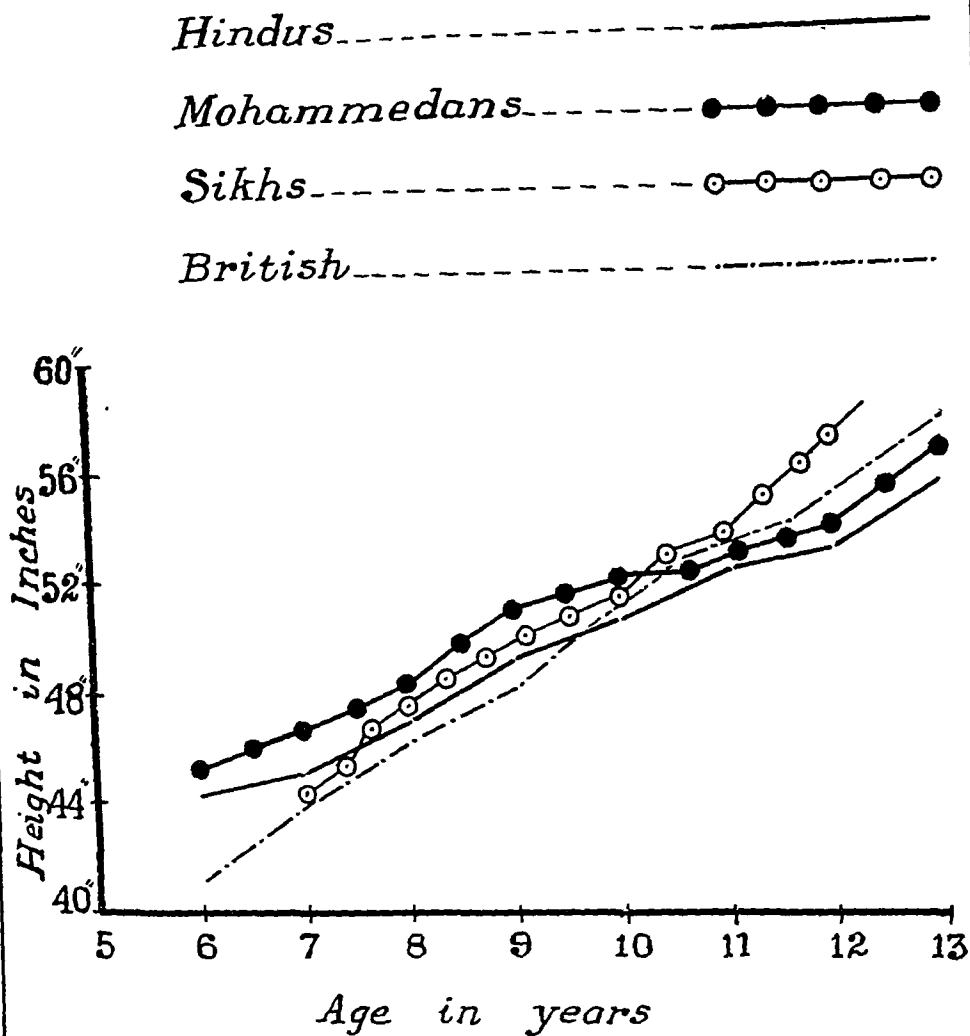


FIG. 10

PUNJAB

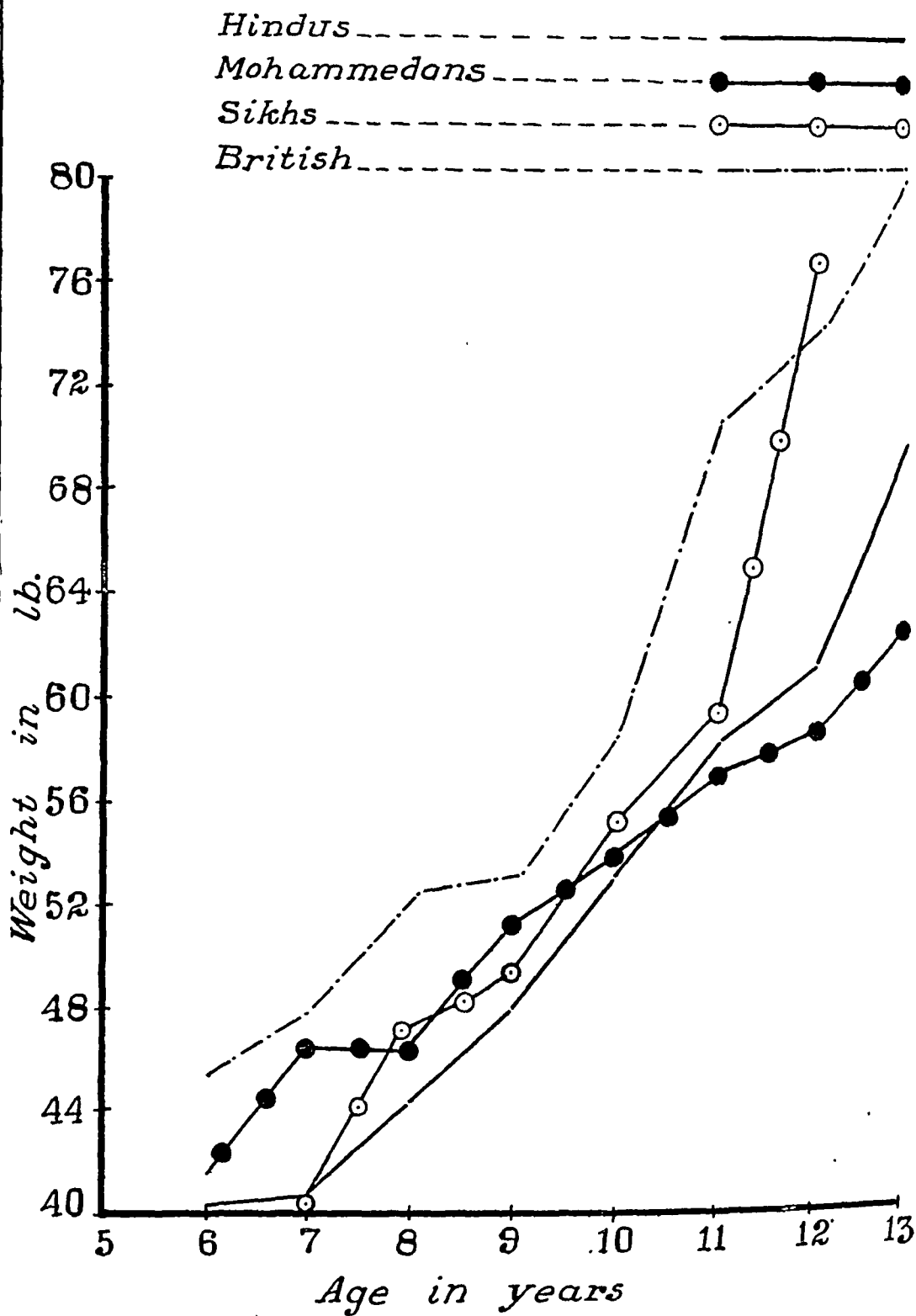


TABLE I.

Rich class Bengali Hindu boys.

SCHOOLS A AND B.					SCHOOL B.	
Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Number of boys.	Caries number.
7	15	48.07	47.71	0	9	7
8	28	48.55	50.20	6	16	3
9	57	51.34	56.19	7	28	11
10	77	52.44	58.31	17	27	9
11	80	53.85	63.40	9	54	12
12	74	56.51	75.04	15	50	6
TOTALS ..	331	..	..	54 (16.3 per cent)	184	48 (26.1 per cent)

TABLE II:

Middle class Bengali Hindu boys.

SCHOOLS C, D, AND E.					SCHOOL C.	
Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Number of boys.	Caries number.
7	28	45.63	42.43	1	18	8
8	64	47.40	44.97	4	39	15
9	84	49.18	49.36	8	38	13
10	127	51.10	53.44	16	79	26
11	110	52.89	59.18	19	68	19
12	88	54.78	64.58	19	47	10
TOTALS ..	501	..	..	67 (13.3 per cent)	289	91 (31.4 per cent)

TABLE III.

Poor class Bengali Hindu boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	CARIES.		ENLARGED TONSILS.		ENLARGED TONSILS + CARIES.	
					Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
7	114	45.43	39.19	0	56	50.0	51	44.7	9	7.8
8	140	47.55	43.45	1	72	51.0	55	32.1	14	10.0
9	104	48.85	46.34	1	41	39.4	41	39.4	7	6.7
10	121	50.63	50.20	5	45	37.1	60	49.9	12	9.9
11	87	53.15	56.23	3	26	29.8	33	37.9	7	8.0
12	32	55.75	64.30	1	7	21.8	19	59.3	3	9.3
TOTALS ..	498	..	..	11 (1.5 per cent)	247	43	259	42	50	8.6

TABLE IV.

Rich class Bengali Mohammedan boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.	Enlarged tonsils number.	Enlarged tonsils + caries number.
7	2	46.65	42.87	0	2	1	1
8	5	46.55	46.20	0	2	1	1
9	12	50.55	49.40	1	6	2	0
10	34	53.03	59.03	2	5	12	2
11	30	54.50	60.85	7	9	6	3
12	33	57.02	69.37	13	2	8	1
TOTALS ..	116	..	..	23 (19.8 per cent)	26 (22.4 per cent)	30 (25.8 per cent)	8 (6.9 per cent)

TABLE V.

Poor class Bengali Mohammedan boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.	Enlarged tonsils number.	Enlarged tonsils + caries number.
7	58	45.15	39.10	1	17	33	14
8	40	43.39	40.80	0	15	21	7
9	42	48.95	45.70	2	10	17	2
10	45	50.84	50.78	1	15	13	4
11	37	52.62	55.45	1	8	10	4
12	28	55.97	62.88	2	4	6	1
TOTALS ..	250	..	..	7 (2.8 per cent)	69 (27.6 per cent)	100 (40 per cent)	32 (12.8 per cent)

TABLE VI.

Rich class Marwari boys.

Age groups.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.	Enlarged tonsils number.	Enlarged tonsils + caries number.
7	14	44.75	41.60	0	4	3	0
8	17	46.55	45.20	0	8	6	3
9	14	49.05	50.20	0	4	3	1
10	48	50.90	54.93	1	8	7	2
11	69	53.30	62.11	5	13	19	4
12	79	53.85	64.30	4	16	24	10
TOTALS ..	241	..	..	10 (4.1 per cent)	53 (21.9 per cent)	62 (25.7 per cent)	20 (8.3 per cent)

TABLE VII.

Poor class Marwari boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.	Enlarged tonsils number.	Enlarged tonsils + caries number.
7	29	45.77	40.33	0	6	17	5
8	33	48.25	45.57	1	19	21	15
9	31	50.54	51.62	0	12	9	5
10	32	52.75	56.86	0	8	14	4
11	4	57.05	70.50	0	0	2	0
12	9	54.78	61.75	0	4	3	3
TOTALS ..	138	..	..	..	49 (35.5 per cent)	66 (47.8 per cent)	32 (23.1 per cent)

TABLE VIII.

Rich class Anglo-Indian boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.
7	8	47.71	45.81	0	3
8	17	48.53	50.60	1	8
9	26	50.45	52.70	0	16
10	31	52.48	58.03	2	12
11	31	52.70	64.27	1	8
12	29	56.15	70.00	1	8
TOTALS ..	142	..	..	5 (3.5 per cent)	55 (38.7 per cent)

TABLE IX.

Poor class Anglo-Indian boys.

Age group.	Number of boys.	Average height in inches.	Average weight in lb.	Number selected by A. C. H. index.	Caries number.
7	6	47.25	42.3	0	4
8	26	47.95	45.9	0	20
9	18	48.88	46.5	2	10
10	23	50.75	52.2	0	14
11	22	52.65	57.41	0	8
12	11	54.35	61.6	2	4
TOTALS ..	106	..	..	4 (3.7 per cent)	60 (56.6 per cent)

TABLE X.

Analysis of Ferozepore (Punjab) data.

Community.	Number of children.	Enlarged tonsils per cent.	Caries per cent.	PER CENT SELECTED BY A. C. H. INDEX FROM					
				Total group.	Good physique.	Fair physique.	Moderate physique.	Poor physique.	Those taking milk.
Punjab Hindus ..	642	26.2	38.3	5.6	4.4	5.0	2.9	28.7	4.1
Punjab Mohammedans ..	465	24.9	30.5	10.1	14.8	8.6	7.8	30.5	1.3
Punjab Sikhs ..	144	27.0	36.8	6.9	8.6	0	4.4	40.0	5.7

TABLE XI.

Analysis of Calcutta data.

Community.	Enlarged tonsils per cent.	Caries per cent.	Enlarged tonsils and caries per cent.	Number selected by A. C. H. index.
Bengali Hindus : Rich class ..	..	26.2	..	16.3
„ „ Middle class ..	..	31.4	..	13.3
„ „ Poor class ..	42.0	43.0	8.6	1.5
„ Mohammedans : Rich class ..	25.8	22.4	6.9	19.8
„ „ Poor class ..	46.0	27.6	12.8	2.8
Marwaris : Rich class ..	25.7	21.9	8.3	4.1
„ (Hindustani) : Poor class	47.8	35.5	23.1	1.0
Anglo-Indians : Rich class ..	..	38.7	..	3.5
„ „ Poor class ..	..	56.6	..	3.7

DISCUSSION.

Taken all over, the children examined in Calcutta and Ferozepore (Punjab) do not show any signs, as far as present clinical experience goes, of malnutrition due to a gross lack of balance in the diet, particularly in regard to the protective foods. That a defective diet exists, however, in the Calcutta children is shown only too clearly in the heights and weights of the different economic classes of each community. In Ferozepore, if the clinical assessment of the children, taken in conjunction with the higher figure for those selected by the index among those children arbitrarily assessed as of poor physique, is correct, the incidence of malnutrition may be no higher than would have been selected in the U. S. A. It should be pointed out also that the lower figure given by the index among those taking milk regularly, favours the assumption that the physique might be better were the diet improved.

As regards the nature of the deficiency in Calcutta, the observations recorded here, when considered along with our knowledge of the diets consumed in the town, would seem to warrant the conclusion that the main deficiency is one of protein primarily and fat to a certain degree. Experience in Calcutta shows that a deficiency of calories is not marked among the Indian communities but in the poor Anglo-Indian class there was strong evidence obtained from home conditions that there was often a gross lack in the quantity of food, even bread, consumed. As far as the observation extends in Calcutta, the A. C. H. index does not appear to be applicable. Apparently those of the poorer classes are built on an all-round smaller scale, so that there is no disproportion between muscle and bone. It is for this reason that we incline to the view that lack of the proximate principle protein is the main deficiency. It is also likely that a special set of standard figures will have to be made for the index, if it is to apply to India, and possibly also for the different races. The negative findings with the index in Calcutta, however, in view of what it purports to measure and in view also of the fact that malnutrition does exist, have been of value, in our opinion, in suggesting or rather emphasizing a deficiency, namely protein, which was observed and commented on many years ago by McKay (1912) in this town.

In view of the fact that protein is an essential building material for body tissue without which, even in the presence of an abundance of vitamins, normal growth could not be completed, it appears to us advisable to stress this point particularly in propaganda work where the lay public possibly tend to think of the vitamins to the exclusion of other nutriment. It should also be pointed out that in many cases the exact vitamin requirements can be expressed in quantitative terms, whereas no such figure has yet (based on any objective criterion) been given for protein. A general agreement has been arrived at as a safe or optimum protein intake but there can be no doubt that the majority of the people of India have a protein consumption which falls short of any authoritative standard for the foodstuff.

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TABLE X.

Analysis of Ferozepore (Punjab) data.

Community.	Number of children.	Enlarged tonsils per cent.	Caries per cent.	PER CENT SELECTED BY A. C. H. INDEX FROM					
				Total group.	Good physique.	Fair physique.	Moderate physique.	Poor physique.	Those taking milk.
Punjab Hindus ..	642	26.2	38.3	5.6	4.4	5.0	2.9	28.7	4.1
Punjab Moham- medans ..	465	24.9	30.5	10.1	14.8	8.6	7.8	30.5	13
Punjab Sikhs ..	144	27.0	36.8	6.9	8.6	0	4.4	40.0	5.7

TABLE XI.

Analysis of Calcutta data.

Community.	Enlarged tonsils per cent.	Caries per cent.	Enlarged tonsils and caries per cent.	Number selected by A. C. H. index.
Bengali Hindus: Rich class ..	..	26.2	..	16.3
„ „ Middle class ..	..	31.4	..	13.3
„ „ Poor class ..	42.0	43.0	8.6	1.5
„ Mohammedans: Rich class ..	25.8	22.4	6.9	19.8
„ „ Poor class ..	46.0	27.6	12.8	2.8
Marwaris: Rich class ..	25.7	21.9	8.3	4.1
„ (Hindustani): Poor class	47.8	35.5	23.1	1.0
Anglo-Indians: Rich class ..	..	38.7	..	3.5
„ „ Poor class ..	..	56.6	..	3.7

ASCORBIC-ACID OXIDASE IN PLANT AND ANIMAL TISSUES.

BY

R. K. CHAKRABORTY,

AND

B. C. GUHA.

(From the Indian Institute for Medical Research, Calcutta, and the University College of Science, Calcutta.)

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SZENT-GYÖRGYI (1930, 1931) has shown that cabbage leaf contains an enzyme which oxidizes ascorbic acid at a very high rate. Tauber, Kleiner and Mishkind (1935) have obtained concentrated ascorbic-acid oxidase preparations from Hubbard squash. The present piece of work was undertaken in order to obtain information about the general distribution of the enzyme in various fruits, vegetables and animal tissues, necessary for the further investigation of the enzyme.

EXPERIMENTAL.

I. Plant tissues.

The fruits and the vegetables with which the experiments were performed were all obtained from the local market. Four or five fruits or vegetables of each variety were cut up into pieces, mixed up and 20 g. from the sample were taken, ground well in a mortar with a small amount of sea-sand, shaken gently for 5 minutes with 60 c.c. of 30 per cent alcohol in a 500 c.c. flask and filtered and the filtrate was used as the source of the enzyme (see Tauber, Kleiner and Mishkind, *loc. cit.*).

In order to test for the enzyme in the alcoholic extract, the solutions were taken in the manner mentioned below in three conical flasks of the same capacity provided with rubber corks:—

- (1) Ten c.c. of the alcoholic extract + 3 c.c. of ascorbic-acid solution (containing a known amount of ascorbic acid) + 7 c.c. of acetate buffer of pH 5.6.

(2) Ten c.c. of the alcoholic extract + 10 c.c. of the buffer of pH 5.6.

(3) Three c.c. of ascorbic-acid solution [same solution as that used in flask (1)] + 6 c.c. of 50 per cent alcohol + 11 c.c. of the buffer of pH 5.6.

No. (1) represents the enzyme solution with ascorbic acid; No. (2) provides the control with the enzyme solution without ascorbic acid; and No. (3) provides the control with ascorbic acid without the enzyme. The total volume is 20 c.c. in each case and contains the same percentage of alcohol.

The flasks were incubated in a thermostat at 38°C. for 45 minutes in all experiments. After the period of incubation, they were taken out of the thermostat, and 1 c.c. of a 2 per cent sulphuric-acid solution was added to each of the flasks in order to stop the activity of the enzyme, if any. Then the amount of ascorbic acid in each of the flasks was estimated by the usual titrimetric technique with the indophenol indicator (Ghosh and Guha, 1935). The total amount of ascorbic acid present in flasks (2) and (3) *minus* the amount of ascorbic acid present in flask (1) after incubation gives the amount of ascorbic acid oxidized by the enzyme.

Figures are given in the Table.

II. Animal tissues.

A known weight of animal tissue (usually 14 g. to 16 g.) was taken in a mortar, ground well with sea-sand, shaken gently for 5 minutes with 30 per cent alcohol in a flask and then centrifuged. The centrifugate was used as the source of the enzyme. For 1 g. tissue, 3 c.c. of the 30 per cent alcohol were used. The amount of ascorbic acid taken was 3 mg. in a total volume of 20 c.c. in each case.

TABLE.

Serial number.	Bengali names.	English names.	Botanical names.	Amount of ascorbic acid taken (in mg.).	Amount of ascorbic acid (in mg.) oxidized by the enzyme under stated conditions.
1	Anarash ..	Pineapple ..	<i>Ananas sativa</i> ..	3.0	0
2	Peara	Guava ..	<i>Spidium gujava</i> ..	3.0	0
3	Mung (not germinated).	Black gram ..	<i>Phaseolus mungo</i> ..	3.0	0
4	Chhola (not germinated).	Bengal gram ..	<i>Cicer arietinum</i> ..	3.0	0
5	Dhenrôs ..	Lady's fingers..	<i>Hibiscus esculentus</i> ..	2.0	0
6	Peauj	Onion ..	<i>Allium cepa</i> ..	3.0	0
7	Rasun ..	Garlic ..	<i>Allium sativum</i> ..	1.2	0

TABLE—contd.

Serial number.	Bengali names.	English names.	Botanical names.	Amount of ascorbic acid taken (in mg.).	Amount of ascorbic acid (in mg.) oxidized by the enzyme under stated conditions.
8	Mula	Radish ..	<i>Raphanus sativus</i> ..	3.0	0
9	Matar-shuti ..	Peas (green) ..	<i>Pisum sativum</i> ..	2.0	0
10	Shalgom ..	Turnip ..	<i>Brassica campestris</i> , var.	2.0	0
11	Tomato ..	Tomato ..	<i>Lycopersicum esculentum</i>	2.0	0
12	Batapi lebu ..	Shaddock ..	<i>Citrus decumana</i> ..	1.5	0
13	Lebu ..	Lemon ..	<i>Citrus medica</i> ..	1.5	0
14	Kamala lebu ..	Orange ..	<i>Citrus aurantium</i> ..	1.5	0
15	Paniphal ..	Water chestnut	<i>Trapa bispinosa</i> ..	1.8	0
16	Ata ..	Custard apple..	<i>Anona squamosa</i> ..	1.8	0
17	Pepe (green) ..	Papaya ..	<i>Carica papaya</i> ..	1.5	0
18	Tal ..	Palm ..	<i>Borassus flabellifer</i> ..	1.8	0
19	Am ..	Mango ..	<i>Mangifera indica</i> ..	3.0	0
20	Narikel ..	Coco-nut ..	<i>Cocos nucifera</i> ..	1.8	0
21	Bhutta ..	Maize ..	<i>Zea mays</i> ..	1.8	0
22	Angur ..	Grape ..	<i>Vitis vinifera</i> ..	1.2	0.10
23	Chhola (germinated)	Bengal gram ..	<i>Cicer arietinum</i> ..	1.5	0.20
24	Palong sak ..	Spinach ..	<i>Spinach oleracea</i> ..	3.0	0.23
25	Bel ..	Wood apple ..	<i>Aegle marmelos</i> ..	3.0	0.25
26	Kancha lanka ..	Green chillies..	<i>Capsicus indicus</i> ..	3.0	0.28
27	Puin sak ..	— ..	<i>Bassella cardifolia</i> ..	2.0	0.32
28	Apel ..	Apple ..	<i>Pyrus molus</i> ..	3.0	0.38
29	Kala (Singapuri) ..	Banana ..	<i>Musa sapientum</i> ..	2.0	0.38
30	Kala (Martaman) ..	Banana ..	<i>Musa sapientum</i> ..	2.0	0.38
31	French beans ..	French beans..	<i>Phaseolus vulgaris</i> ..	3.0	0.42
32	Nespati ..	Pears (country)	<i>Pyrus communis</i> ..	3.0	0.57

TABLE—concl'd.

Serial number.	Bengali names.	English names.	Botanical names.	Amount of ascorbic acid taken (in mg.).	Amount of ascorbic acid (in mg.) oxidized by the enzyme under stated conditions.
33	Dalim	Pomegranate ..	<i>Punica granatum</i> ..	1.8	0.66
34	Aloo	Potato ..	<i>Solanum tuberosum</i> ..	3.0	1.16
35	Kalmi sak ..	Ipomœa ..	<i>Ipomœa reptans</i> ..	2.0	1.27
36	Mung (germinated)	Gram ..	<i>Phaseolus mungo</i> ..	3.0	1.41
37	Begoon ..	Brinjal ..	<i>Solanum melongena</i> ..	3.0	1.46
38	Fulkopi ..	Cauliflower ..	<i>Brassica oleracea</i> , var. <i>botrytis</i> .	3.0	1.59
39	Misti kumro ..	Sweet gourd ..	<i>Cucurbita maxima</i> ..	3.0	1.84
40	Gajar	Carrot ..	<i>Daucus carota</i> ..	3.0	1.86
41	Patôl	— ..	<i>Trichonasthes dioica</i> ..	3.0	1.91
42	Jhinge ..	Ridge gourd ..	<i>Luffa acutangula</i> ..	3.8	2.04
43	Lettuce ..	Lettuce ..	<i>Lactuca sativa</i> ..	3.0	2.22
44	Lau	Gourd ..	<i>Lagenaria vulgaris</i> ..	3.0	2.29
45	Hinche ..	— ..	<i>Enhydra fluctuans</i> ..	3.0	2.34
		White gourd ..	<i>Benincasacriapra</i> ..	3.0	2.76
		Cucumb. ..	<i>Cucumis sativus</i> ..	5.0	2.94

— were obtained from the local slaughter house and the
 .. Pineapple 3 to 6 hours after slaughtering of the animal. The
 .. Guava obtained by killing them in the laboratory and the
 .. immediately. The fish-liver was obtained from the
 .. Black
 .. ts with p^l animal tissues, a known weight of
 .. Benga solution his was diluted to a definite volume
 .. frigerat^l phuretted hydrogen was passed into
 .. Lady's e any y oxidized ascorbic acid which might
 .. sol phuretted hydrogen was driven off by
 .. Onion
 .. Garlic s
 .. s in any of the animal tissues investigated,
 .. w, guinea-pig, rabbit and fowl; the liver

tissue of the *katla* fish (*Catla catla*) and the kidney tissue of the rat; the adrenal, pituitary, brain and pancreas of the cow.

DISCUSSION.

In this work we have not made a strictly quantitative comparison among the substances investigated, as the concentration of ascorbic acid used was not constant in all experiments and conditions with reference to the presence of metallic catalysts and other interfering substances, which might influence the oxidation of ascorbic acid, could not possibly be the same. Attempts will be made later to fix the conditions for more quantitative comparisons. The results indicate, however, that among the foodstuffs investigated, Sasha (*Cucumis sativus*), Chal kumro (*Benincasacripra*), Hinche (*Enhydra fluctuans*), Lau (*Lagenaria vulgaris*), Lettuce (*Lactuca sativa*), Jhinge (*Luffa acutangula*), Patôl (*Trichonasthes dioica*), Gajar (*Daucus carota*), Misti kumro (*Cucurbita maxima*), Fulkopi (*Brassica oleracea*, var. *Botrytis*) and Begoon (*Solenum melongena*) contain a fair amount of ascorbic-acid oxidase, Sasha (*Cucumis sativus*) and Chal kumro (*Benincasacripra*) being the richest sources among these. It is interesting to note that germination produces ascorbic-acid oxidase in Kancha mung (*Phaseolus mungo*) just as it produces ascorbic acid.

In the liver and kidney of different species, such as the cow, guinea-pig, rabbit, fish, and fowl, no ascorbic-acid oxidase could be detected. The adrenal, pituitary, brain and pancreas of the cow were also examined but none of them contained the enzyme. These results are in marked contrast to those obtained with plant tissues many of which contain fair amounts of the oxidase.

SUMMARY.

(1) A large number of plant and animal tissues have been investigated with reference to their contents of ascorbic-acid oxidase.

(2) Among these Sasha (*Cucumis sativus*) seems to be the richest source of the enzyme.

(3) Germination leads to the formation of ascorbic-acid oxidase in Kancha mung (*Phaseolus mungo*).

(4) The animal tissues investigated, in contrast to most of the plant tissues, seem to be completely devoid of the enzyme.

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animals were estimated and also 30, 60, 90, and 120 minutes after the injection of glucose. The figures are shown in Table I.

- (b) The experiments were performed in the same way as before. This group of animals received only an injection of 0.5 c.c. adrenalin chloride. The results of the estimation of blood sugar and calcium are shown in Table II. There was apparently an increase in the sugar content of the blood but no change in the calcium content.
- (c) This group was also treated in the same way as group (a), except that adrenalin was injected immediately after the injection of glucose. The results of estimation of blood sugar and calcium have been shown in Table III. It is seen that, while there was a rise in sugar, there was no change in the calcium content of the blood.
- (d) This group of animals was also treated in the same way as above except that adrenalin chloride was injected half an hour after glucose. The figures for blood calcium and sugar are given in Table IV.
- (e) This group of animals was also treated as in the case of the other groups but they received an injection of adrenalin half an hour previous to the administration of glucose. The results of the estimation are to be found in Table V. In these animals there was no rise of calcium.

It was noticed that the administration of glucose was followed by an increase of titrable alkali of blood. For titration 1.5 c.c. of serum of the treated animal was diluted with 1.5 c.c. of distilled water and titrated with N/50 HCl using cresol red as indicator. This increase of titrable alkali has been shown in Tables I and IV.

Injections of alkali, however, did not increase the calcium content of blood.

The result of the experiments may be summarized in the following table:—

	Calcium content of blood.	Glucose content of blood.	Titrable alkali in blood.
1. Adrenalin injection.	No change.	Increase.	Not investigated.
2. Adrenalin and glucose injected successively.	„	Marked increase.	No change.
3. Glucose and adrenalin injected successively.	„	Increase.	„
4. Adrenalin injected half an hour after glucose.	Increase in calcium but after adrenalin is injected it is restored to normal.	„	Increase at the beginning but later comes down to normal along with calcium.
5. Alkali solution injected in a small quantity.	No change.	No change.	No perceptible change.

TABLE I.

Weight of rabbits in g.	AFTER INJECTION OF GLUCOSE.															
	BEFORE INJECTION.				30 MINUTES.			60 MINUTES.			90 MINUTES.			120 MINUTES.		
	Doses of glucose injected in c.c.	Calcium in mg. per cent.	Sugar per cent.	Alkali in c.c.	Calcium in mg. per cent.	Sugar per cent.	Alkali in c.c.	Calcium in mg. per cent.	Sugar per cent.	Alkali in c.c.	Calcium in mg. per cent.	Sugar per cent.	Alkali in c.c.	Calcium in mg. per cent.	Sugar per cent.	Alkali in c.c.
985 ..	0.9	12.0	0.066	1.05	16.0	0.111	1.20	16.0	0.117	1.20	16.5	0.117	1.20	16.0	0.166	1.20
1,360 ..	1.3	16.0	0.086	1.20	16.0	0.133	1.20	18.0	0.153	1.35	18.5	0.181	1.35	18.0	0.181	1.35
1,115 ..	1.1	11.2	0.080	1.20	12.5	0.111	1.35	13.2	0.117	1.35	13.2	0.181	1.35	14.0	0.200	1.35
1,125 ..	1.1	12.0	0.090	1.05	13.5	0.133	1.20	14.0	0.181	1.35	16.0	0.200	1.50	16.0	0.200	1.50
1,320 ..	1.3	11.0	0.083	0.90	11.5	0.133	1.05	12.0	0.166	1.05	14.2	0.166	1.05	15.0	0.181	1.20
1,400 ..	1.4	11.2	0.076	0.90	11.0	0.105	0.90	13.2	0.117	1.05	14.0	0.117	1.20	14.5	0.166	1.20
1,168 ..	1.1	11.5	0.074	0.90	11.5	0.105	0.90	14.2	0.111	1.05	15.0	0.166	1.05	15.0	0.166	1.20
1,210 ..	1.2	12.0	0.080	0.90	14.0	0.117	1.05	16.0	0.117	1.20	15.8	0.133	1.20	15.5	0.181	1.20

TABLE II.

BEFORE INJECTION.			AFTER INJECTION OF ADRENALIN.							
			30 MINUTES.		60 MINUTES.		90 MINUTES.		120 MINUTES.	
			Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.
1	14.0	0.086	13.9	0.125	14.1	0.181	14.0	0.200	14.0	0.250
2	12.0	0.080	12.2	0.086	12.0	0.125	12.1	0.133	12.0	0.181
3	13.0	0.083	13.0	0.086	13.0	0.105	12.9	0.125	13.0	0.181
4	17.0	0.105	17.0	0.125	17.1	0.181	17.0	0.200	17.0	0.250
5	14.0	0.080	14.2	0.090	14.0	0.133	14.0	0.166	14.0	0.181
6	12.0	0.083	12.0	0.095	12.1	0.105	12.0	0.133	12.0	0.153
7	13.2	0.086	13.0	0.117	13.2	0.153	13.0	0.181	13.0	0.250
8	14.0	0.083	14.0	0.100	14.0	0.125	14.2	0.142	14.2	0.166

TABLE III.

Weight of rabbits in g.	BEFORE INJECTION.			SUGAR INJECTION IMMEDIATELY FOLLOWED BY ADRENALIN.							
				30 MINUTES.		60 MINUTES.		90 MINUTES.		120 MINUTES.	
	Doses of glucose injection in c.c.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.	Calcium in mg. per cent.	Sugar per cent.
1,368 ..	1.3	16.0	0.083	16.0	0.090	15.9	0.105	16.0	0.181	16.0	0.200
1,062 ..	1.0	11.2	0.086	11.2	0.100	11.2	0.153	11.0	0.181	11.2	0.250
1,120 ..	1.1	13.0	0.074	13.0	0.100	13.1	0.133	13.0	0.166	13.0	0.181
1,420 ..	1.4	12.2	0.076	12.0	0.105	12.0	0.153	12.2	0.181	12.2	0.200
1,400 ..	1.4	12.0	0.080	12.0	0.117	12.2	0.166	12.0	0.200	12.0	0.250
1,170 ..	1.1	13.0	0.083	13.0	0.133	13.0	0.181	13.0	0.181	13.0	0.200
1,210 ..	1.2	16.2	0.076	16.2	0.111	16.2	0.181	16.2	0.200	16.2	0.250
1,420 ..	1.4	11.5	0.080	11.5	0.117	11.5	0.153	11.5	0.181	11.5	0.200

TABLE IV.

Weight of rabbits in g.	BEFORE INJECTION.				30 MINUTES AFTER GLUCOSE INJECTION.			INJECTION OF ADRENALIN AFTER 30 MINUTES OF GLUCOSE INJECTION.					
	Doses of glucose injection in c.c.	Calcium in mg. per cent.	Alkali in c.c.	Sugar per cent.	Calcium in mg. per cent.	Alkali in c.c.	Sugar per cent.	30 MINUTES.			60 MINUTES.		
								Calcium in mg. per cent.	Alkali in c.c.	Sugar per cent.	Calcium in mg. per cent.	Alkali in c.c.	Sugar per cent.
1,210 ..	1.2	16.0	0.90	0.065	18.0	1.05	0.166	18.0	1.05	0.166	16.0	0.90	0.181
1,300 ..	1.3	14.0	1.05	0.090	16.6	1.20	0.111	16.0	1.20	0.166	15.0	1.05	0.200
1,000 ..	1.0	13.5	0.90	0.086	16.6	0.90	0.166	14.5	1.05	0.181	14.0	1.05	0.200
900 ..	0.9	12.0	0.90	0.074	14.5	1.20	0.111	13.5	1.05	0.153	13.5	0.90	0.181
1,540 ..	1.5	14.0	1.20	0.083	16.0	1.20	0.166	15.2	1.20	0.181	14.0	1.20	0.200
1,500 ..	1.5	15.0	0.90	0.080	17.0	1.05	0.111	15.0	0.90	0.153	15.0	1.05	0.166
980 ..	0.9	12.0	1.05	0.086	16.0	1.05	0.133	14.0	0.90	0.143	14.0	1.05	0.181
1,400 ..	1.4	11.0	0.90	0.076	14.0	1.05	0.133	14.0	0.90	0.153	13.0	1.05	0.200

TABLE V.

Weight of rabbits in g.	BEFORE INJECTION.		ADRENALIN INJECTION FOLLOWED BY GLUCOSE AFTER AN INTERVAL OF 30 MINUTES.			
	Doses of glucose injection in c.c.	Calcium in mg. per cent.	30 MINUTES Calcium in mg. per cent.	60 MINUTES Calcium in mg. per cent.	90 MINUTES Calcium in mg. per cent.	120 MINUTES Calcium in mg. per cent.
910 ..	0.9	12.0	12.2	11.9	12.0	12.0
1,220 ..	1.2	12.5	12.5	12.15	12.3	12.5
1,200 ..	1.2	11.0	11.2	11.0	11.2	11.0
1,110 ..	1.1	14.0	14.2	14.0	14.1	14.0
1,020 ..	1.0	12.0	12.1	12.0	12.0	12.0
1,050 ..	1.0	13.0	13.0	13.1	13.0	13.0
1,115 ..	1.1	12.0	12.0	12.0	12.0	12.0
1,120 ..	1.1	11.1	11.0	11.0	11.0	11.0

TABLE VI.

Weight of rabbits in g.	BEFORE INJECTION.			AFTER INJECTION OF GLUCOSE 30 MINUTES.	
	Doses of injection in c.c.	Phosphate in mg. per cent.	Calcium in mg. per cent.	Phosphate in mg. per cent.	Calcium in mg. per cent.
1,120 ..	1.1	5.0	12.0	4.1	14.0
1,010 ..	1.0	4.2	11.5	3.8	16.0
1,020 ..	1.0	4.0	13.0	3.5	14.2
1,510 ..	1.5	4.0	12.0	4.0	12.5
1,035 ..	1.0	3.5	11.2	2.8	14.0
1,260 ..	1.2	4.0	11.0	3.2	14.0
1,361 ..	1.3	3.0	12.0	3.0	12.5

DISCUSSION.

It is evident from the above results that adrenalin exercises an inhibitory action on the increase of blood calcium after administration of glucose intravenously. It has been shown by various workers that the calcium content of blood is normally regulated by the hormone of parathyroid, vitamin D, and the phosphates of blood (Read, 1928). The influence of vitamin D in the increase of calcium in our experiments can be discarded, as all the animals were given the same food. The phosphates of blood shown in Table VI were found to have decreased whenever there was increase in calcium.

We know that increase of glucose in blood in all normal conditions of the organism stimulates increased insulin secretion. Again, pancreas and parathyroids are known to co-operate with one another regarding various metabolic activities of the body. It may, therefore, be assumed that glucose causes an increase of blood calcium through the influence of pancreas on parathyroids. Adrenal glands and pancreas are antagonistic to each other (Harrower, 1933). Consequently excess of adrenalin in blood may cause inhibition of pancreatic activity. This fact is corroborated by our experiments. It is clear thus that if adrenalin is injected along with glucose or immediately before or after glucose, glucose will not be allowed to exert its influence on pancreas owing to the inhibiting influence of adrenalin on pancreas, and hence there is no rise of calcium. From the above experiments it may be assumed that glucose has a stimulating action on parathyroids either directly or indirectly through pancreas.

Possible factors involved in increasing the alkalinity after administration of glucose :—

It was noticed that glucose increased the calcium content and alkalinity of blood simultaneously as shown in our experiments.

This increase of alkalinity may be attributed to the increase in the bases of blood. Administration of parathyroid also produces a temporary increase in the alkalinity of blood (Cameron, 1933). It is also possible, therefore, that glucose injection directly or indirectly stimulates parathyroid—thus increasing the alkalinity of blood. It is difficult to ascertain definitely which of these two factors is responsible for the increased alkalinity or whether both of these factors are involved in the process.

SUMMARY.

- (1) Glucose injection increases the calcium content of blood.
- (2) Adrenalin injection increases the blood sugar without any change in the calcium content.
- (3) Sugar injection immediately followed by adrenalin does not increase the calcium though blood sugar increases to a great extent.
- (4) If adrenalin be injected after a rise of calcium on glucose injection the curve of calcium gradually comes down to normal.
- (5) If glucose be injected after adrenalin no change of the calcium content takes place.

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HÆMATOLOGICAL STUDIES IN INDIANS.

Part VI.

INVESTIGATIONS IN 100 CASES OF MARKED ANÆMIA AMONGST TEA-GARDEN COOLIES.

BY

L. EVERARD NAPIER, M.R.C.P. (Lond.),

AND

C. R. DAS GUPTA, M.B., D.T.M.

(An Inquiry under the Endowment Fund of the School.)

From the School of Tropical Medicine, Calcutta.

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AN investigation into the cause of the anæmia that is so prevalent amongst tea-garden coolies was commenced by the writers in 1933. Some of the preliminary steps in this investigation, hæmatological examinations in normal city-dwelling Indians and in so-called 'normal' coolies, have been reported in the earlier parts of these studies.

In this paper, we are reporting the blood findings in definite cases of anæmia amongst the labour forces in gardens in the practices of Doctors D. Manson and G. Macdonald. The first series of cases were investigated during the cold weather months of 1933-1934, and the second between November 1934 and May 1935

THE FIRST SERIES.

The clinical material.—Our patients were untreated patients attending the hospitals on certain tea estates in the practices of the two doctors referred to above; most of these patients had been selected during the systematic examination of coolies carried out each year on these estates, but a few had reported sick voluntarily. The selection was made mainly on clinical grounds, that is to say, on appearance or because they complained of some shortness of breath, but a Tallqvist reading of 50 per cent, or less, was taken as the criterion. The clinical features presented by

the patients were fairly constant but do not lend themselves to analysis. Their general appearance was anæmic, with a slight degree of puffiness of the face and the whole body, and œdema of the legs. In a few cases there was obvious general anasarca, but usually the puffiness of the skin on the trunk was only noticeable after prolonged pressure, e.g., with the stethoscope. The tongue had the wash-leather appearance, but was never red or sore. Most of the patients were thin but not emaciated.

There was usually a hæmic murmur; the lungs were usually normal. The pulse rate was between 90 and 100 in the majority of cases, and the blood pressure low, seldom above 110 mm. Hg. (systolic). There were no constant intestinal symptoms and no nervous symptoms.

Night-blindness was a common complaint but there were few other obvious signs of specific nutritional deficiency, though there was evidence of general under-nourishment. Koilonychia was not noticed.

They were nearly all outdoor labourers, both men and women. None of them were aboriginal inhabitants of Assam, but some had been born on the gardens, nearly all had been in Assam for many years, and none had lived there for less than a year.

They came originally from different parts of India, and were members of innumerable different Hindu castes. They were all good wage earners, and, as the man, the woman and the children all work, there is no danger of want on account of shortage of money, taking the coolies as a whole. Inquiries regarding diet elicited very vague replies; rice is of course the staple diet but most take meat occasionally, once or twice a week. Little milk is taken. It was very difficult to gauge the amount of vegetables and fat consumed. There is certainly reason to believe that the fat consumption is very low. Some fruit is usually taken and nearly all drink an alcoholic country beer which is reputed to be rich in vitamin-B complex.

Age and sex.—There were 26 males and 32 females. The ages varied from 13 to 67 in the first series; grouped by decades they are distributed as follows:—

Decade.	Males.	Females.
10-20 ..	5	11
20-30 ..	8	16
30-40 ..	9	2
40-50 ..	4	2
Over 50 ..	..	1
TOTALS ..	26	32

It will be seen that there is a marked contrast in the sex distribution below and above 30. The difference is statistically very 'significant'.

The age given by coolie patients is, however, always approximate. The same age-sex grouping is not apparent in the second series, but this may be because a number of women with young children were deliberately excluded.

Technique.—The method adopted in the case of the normal persons was again followed. Five c.c. of blood was taken from a vein and placed in a small flask containing 0.01 gramme potassium oxalate as an anti-coagulant, and all examinations except the coagulation time and the reticulocyte counts were made from this specimen.

Hæmoglobin estimation.—A standardized Hellige hæmometer was used and the readings were taken after 20 minutes, as in our earlier work. The results were recorded as grammes per 100 c.c. of blood.

There were originally 35 women and 26 men selected and examined, but, of these, three women have been excluded from this analysis on the grounds that their hæmoglobin was found, when estimated by the more accurate method, to be well within the 'normal' range, being 8.525 g., 9.35 g., and 9.90 g., respectively. If we adopted the rule that the normal range is the mean $\pm$ twice the standard deviation, we should have to exclude four others, amongst them one with a hæmoglobin value of 6.87 g.: this would be absurd. We cannot adopt the view that the figure, 10.03 g., which we found to be the mean of 17 so-called 'normal' women coolies, represents the mean value of the hæmoglobin of healthy females of the same race.

The lowest figure was 2.475 g., the highest 7.95 g., and the mean 4.80 g. The cases are grouped according to the hæmoglobin content of their blood at the first examination, as follows:—

Hæmoglobin in grammes per 100 c.c.	Males.	Females.	TOTALS.
2.0—2.99 ..	2	5	7
3.0—3.99 ..	4	7	11
4.0—4.99 ..	6	7	13
5.0—5.99 ..	6	7	13
6.0—6.99 ..	7	3	10
7.0—7.99 ..	1	3	4
TOTALS ..	26	32	58

Mean corpuscular values.—We made a cell-volume estimation in a number of cases, but, as we had not standardized our method and had not adopted it as a routine examination at that time, we are not including the cell-volume figures of the first series in this report.

The red cell count was, of course, done so that the mean corpuscular hæmoglobin (MCH) could be worked out for each case.

In Chart 1, the MCH in micro-micro-grammes and the hæmoglobin in grammes per 100 c.c. of blood in these 58 cases are shown. The two lines indicate the normal MCH of, respectively, (m^1) the normal city-dwelling male Indians (from Table VI, Part V) and (m^2) the 'normal' tea-garden coolies, male and female (worked out from the figures used in Part III).

This chart shows (i) that there is in this series no obvious correlation between the degree of anæmia and the amount of hæmoglobin in each cell, (ii) that, even judged on the low standard of the coolie population ($m^2 = 22.06 \pm 2.67 \gamma\gamma$), nearly three-quarters of the cases are hypochromic, and (iii) that judged on normal Indian standards ($m^1 = 28.53 \pm 2.31 \gamma\gamma$) only two can be said to be hyperchromic.

If we take the line at 20 $\gamma\gamma$ as an arbitrary division, it will be seen that there is a marked concentration of cases below this line and that beyond it they are scattered.

This provides an opportunity to separate the cases into two groups—definitely hypochromic and orthochromic, the latter including two cases that come just beyond the normal limits and therefore might be considered as hyperchromic. We will consider the rest of the data, as a whole and in each of our arbitrary and provisional groups, to see if we can justify this division.

Sex.—In the hypochromic series there are 22 males and 20 females: in the orthochromic series there are 4 males and 12 females. There is thus an apparent sex difference in the two series; this difference is nearly but not quite 'significant'*.

Another way of expressing this difference is by the means of the MCH in the two series; in the males it is 18.5 $\gamma\gamma$ and in the females 19.9 $\gamma\gamma$. This sex difference in the MCH's does not exist in any non-anæmia series we have so far examined.

Diameter of the red cells.—In seven cases selected at random the diameter of 500 cells was measured and the mean and coefficient of variation calculated. These are shown in the table below, arranged according to their size; the MCH in each case is entered to show the correlation between the mean diameters and hæmoglobin

* Wherever the word 'significant' is used, statistical significance of a 0.05 order is implied. To take the case in point, if it could be shown that in a chance distribution a certain degree of female predominance would only occur once in twenty times, that predominance is said to be 'significant'; in this case the chances are a little less than 20 to 1. In this case the calculation is made by the formula:

Standard deviation = $p^o q^o \left(\frac{1}{n} + \frac{1}{n'} \right)$. The difference in the percentage of males in the two series is just less than twice the standard deviation. Had the difference been 2 per cent higher it would have been just twice the standard deviation of the difference and would have therefore been considered to be 'significant'.

content of the cells; this is fairly high ($r = 0.6996$). Two sample curves are shown in Charts 1 and 2.

Serial number.	Mean diameter.	Coefficient of variation.	MCH.
43	5.73 μ	0.1595	12.22 $\gamma\gamma$
42	5.99 μ	0.1487	15.63 $\gamma\gamma$
32	6.21 μ	0.1375	17.56 $\gamma\gamma$
55	6.91 μ	0.1295	17.50 $\gamma\gamma$
44	6.92 μ	..	19.91 $\gamma\gamma$
33	6.93 μ	0.1141	26.66 $\gamma\gamma$
29	7.13 μ	0.1198	18.72 $\gamma\gamma$

Reticulocytes.—These were counted in each case by the method already described (see Part II). The lowest count was 0.7 per cent, the highest 9.6 per cent, and the mean of the whole series was 4.26 per cent, of the 26 males 4.16 per cent, and of the 32 females 4.33 per cent. The mean for the orthochromic series was 4.83 per cent.

The mean reticulocyte count for the normals of the same population was 2.17 per cent. In this series 21 males and 24 females have a reticulocyte percentage above this mean, the remaining 5 and 8, respectively, being below it.

Platelets.—These were counted by the method already described, in all except two cases. The mean of the series of 56 cases was 210,750 per c.mm., of the 25 males 254,080 per c.mm., and of the 31 females 175,807 per c.mm.; the highest count was 735,000 and the lowest 25,000 per c.mm. The mean of the orthochromic series was 153,600 per c.mm. Three males and one female only showed a count above 415,000, the 'normal' mean for the same population.

Abnormal red cells.—Anisocytosis and polychromasia were more or less constant findings. Nucleated red cells, normoblasts or macronormoblasts were noted in 13 cases of which 3 were in the orthochromic series.

Halometric readings.—We attach no importance to these readings (see Part V) but they are reported. The means amongst the males, the females, and the whole series were, respectively, 7.52 μ , 7.42 μ , and 7.48 μ ; in three males and six females the estimations were below the 'normal' mean of 7.05 μ (see Part III). In the orthochromic series the mean was 7.39 μ , that is to say, it was less than the mean of the readings in the hypochromic series, which is a very unlikely contingency.

CHART 1.

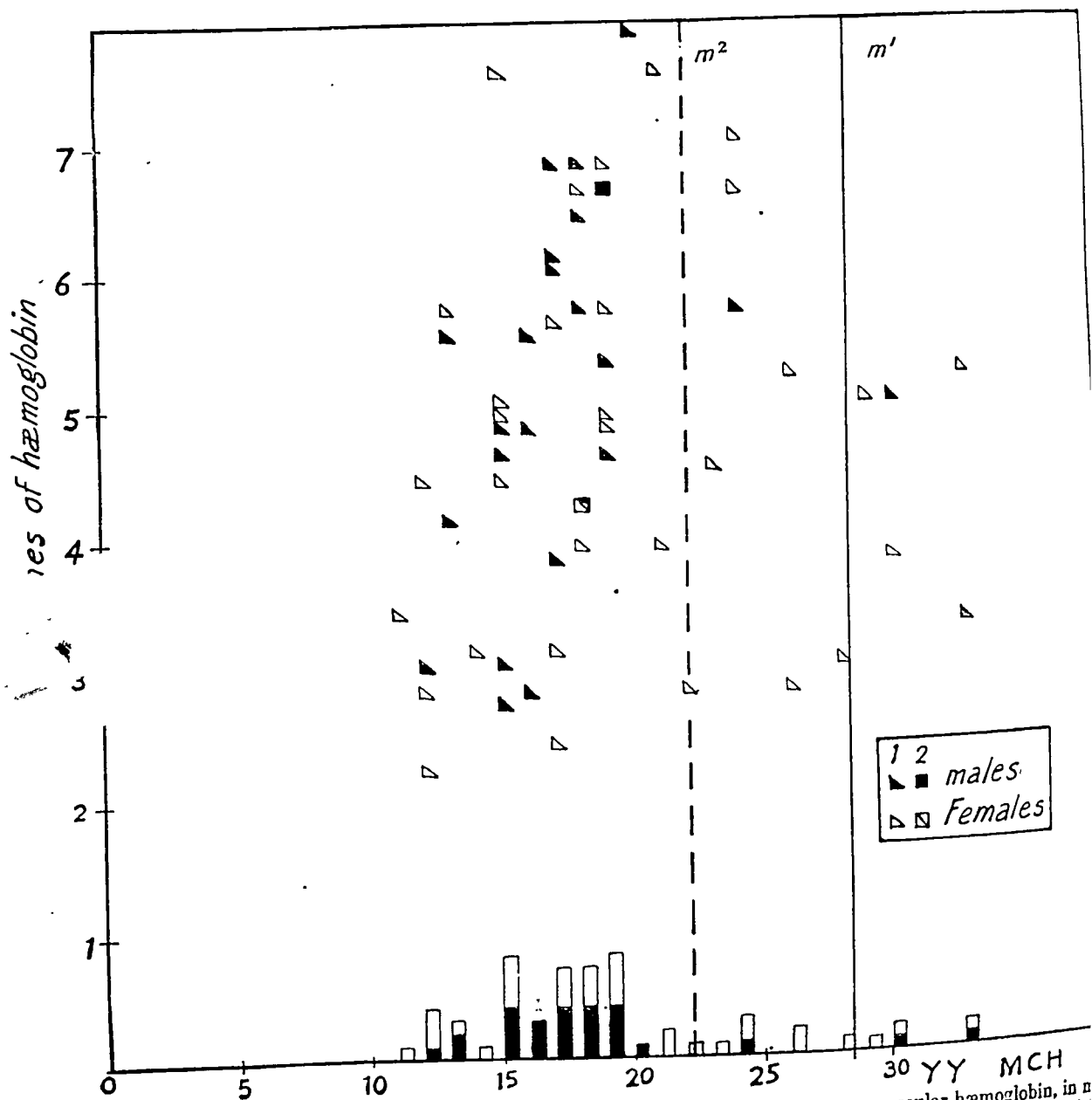


CHART 1. Showing the relationship of the degree of anæmia and the mean corpuscular hæmoglobin, in females; m^1 (line) indicates the normal mean corpuscular hæmoglobin and m^2 (dot line) corpuscular hæmoglobin of the 'normal' coolie population.

CHART 2.

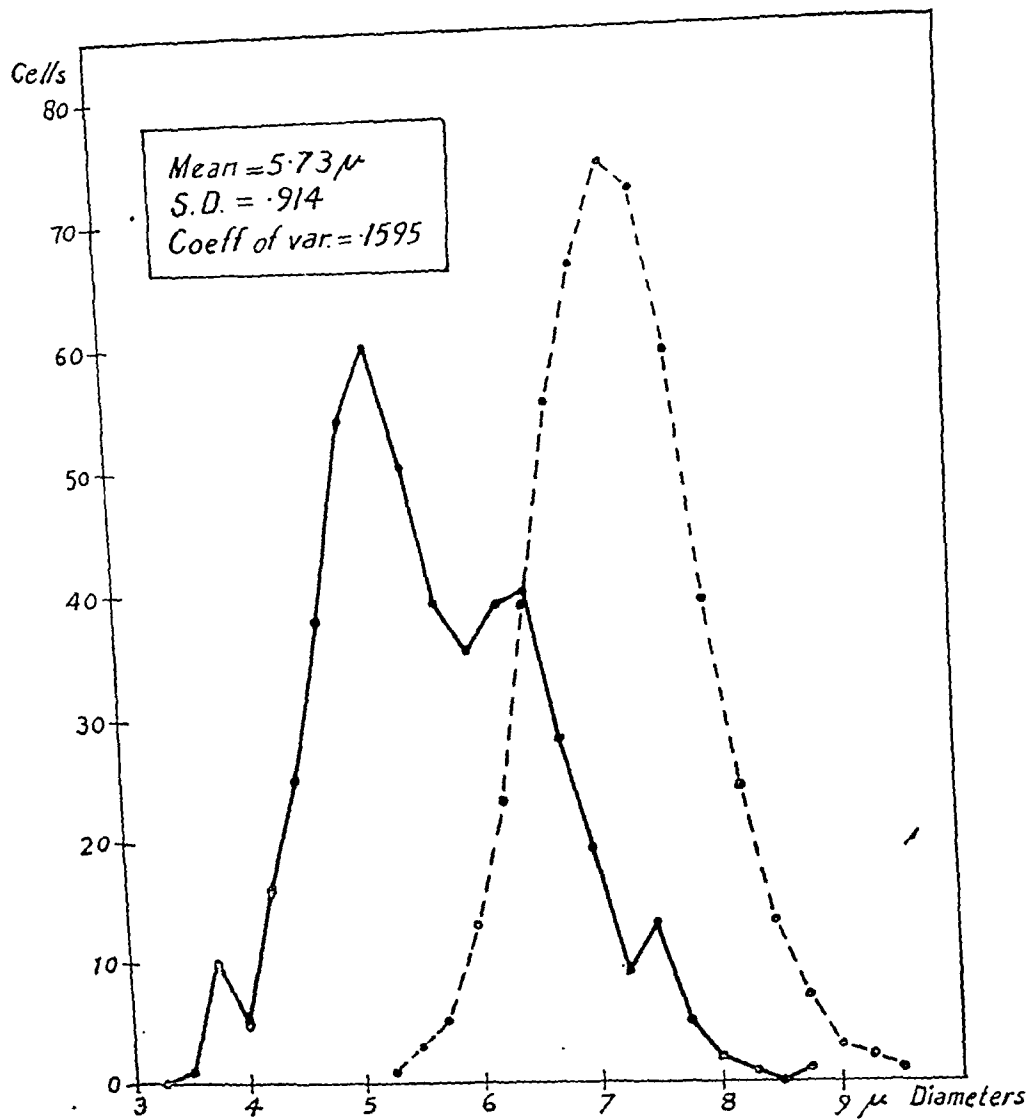


CHART 2. Showing a typical Price-Jones' curve of a hypochromic case; the dot line is a composite curve based on the cell measurements in ten healthy city-dwelling Indians (measured by Dr. P. A. Maplestone).

CHART 3.

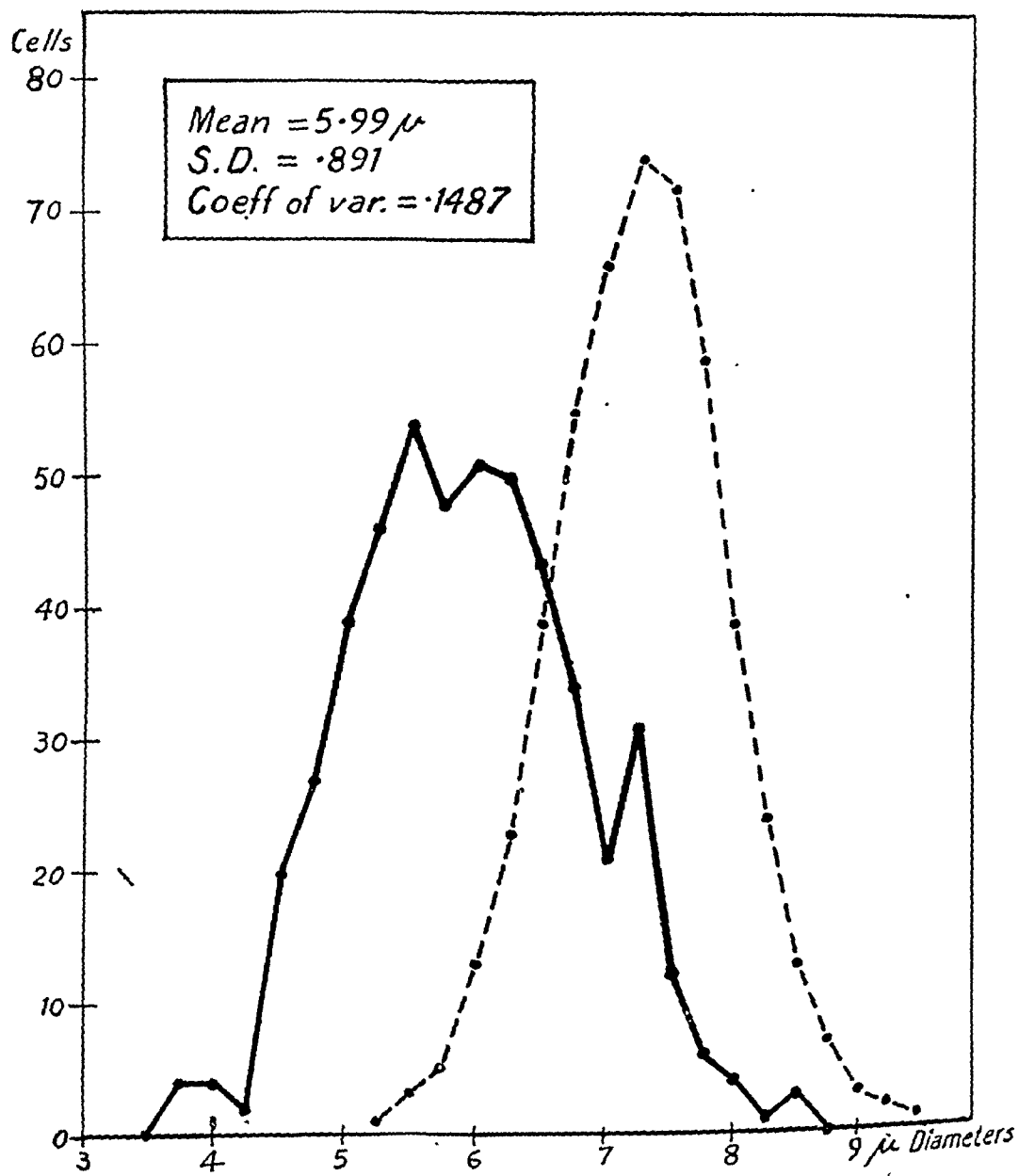


CHART 3. Showing another example of a Price-Jones' curve from a case in this series.

purposes the remainder of the white cells may be considered as having been neutrophil granulocytes.

The fragility test.—This was carried out in the usual way with saline solutions of different concentrations; the strength of the strongest solutions (a) in which slight hæmolysis occurred and (b) in which hæmolysis was complete was noted, and the means of these for males, for females, and for the whole series worked out. The figures, based on 21 males and 30 females, are given below :—

		(a)	(b)
		Hæmolysis commenced, per cent.	Hæmolysis complete, per cent.
Males	..	0.3914	0.2319
Females	..	0.4080	0.2350
TOTALS	..	0.4012	0.2337

These figures indicate that there is little change from the normal in the fragility of the red cells; in no individual case was there any marked departure from the normal.

Coagulation time.—The method used was to take blood into a capillary tube and break the tube at half-minute intervals. We have not made a habit of keeping the tubes in a water-bath, or incubator, as the temperature in Calcutta is so often about blood temperature. We omitted to do this in Assam where the temperature was colder and varied at different periods of the work; the readings must, therefore, be accepted with reserve.

Only 24 were done, 11 males and 13 females. The means of the coagulation times are given below :—

				Mean of readings (minutes).
Males	..	..	..	6.27
Females	..	..	..	6.81
TOTAL	..	..	..	6.58
Standard deviation of mean	..	..	..	6.05

In 35 'normals' the mean was 4.67 ± 2.17 and the difference between males and females was about the same as in this series.

The van den Bergh tests.

In no case was the *direct* test positive. We have always encountered great difficulty in obtaining a solution for matching the van den Bergh results; so much so that we have had to give up all attempts to use the colorimeter and have depended on matching the solution approximately in two test-tubes of the same calibre; we have consequently not used units for indicating the results. We have used the sign (+) to indicate bilirubinæmia of from 0.5 mg. to 1 mg. per 100 c.c., + from 1 mg. to 2 mg., and ++ 2 mg. or over.

The results are shown in the table below:—

TABLE I.

Indirect van den Bergh.	Hypochromic.		Orthochromic.		'Normal' coolies.	
	M.	F.	M.	F.	M.	F.
Negative ..	10	5	1	2	10	2
(+) ..	7	8	1	3	3	..
+	4	7	1	4	1	..
++ ..	1	0	..	2	1	..
		30		7		15
		12		7		2

That is to say, only 12 out of 42 of the hypochromic cases but just half of the orthochromic cases gave a positive van den Bergh.

Van den Bergh and urobilin in the urine.—Urobilin was not noted as being increased unless the fluorescence was very distinct in a dilution of 1 in 20 of urine. Urobilin was present in abnormal amount in the urine of 15 out of 39 of the hypochromic and in 7 out of 12 of the orthochromic cases. The two tests were done in 47 cases:—

	UROBILIN IN URINE.	
	Definitely increased.	Normal.
Positive van den Bergh ..	8	6
Negative van den Bergh ..	13	20

Discussion.—These results are a little surprising for two reasons. There seems to be an unusually high percentage of hyper-bilirubinæmia in the hypochromic cases, in view of the fact that hæmolysis is seldom a feature of this type of anæmia. This led us to carry out the test in a few non-anæmic coolies, as we had unfortunately omitted to do this whilst doing other 'normal' examinations. Circumstances only allowed us to test seventeen 'normal' coolies, of which 15 were men; the results, included in Table I, indicate that there is some imbalance between hæmolysis and utilization of the bilirubin even in these so-called 'normal' people (or there is hepatic dysfunction); if we consider men only the results are not significantly different from those of the hypochromic anæmics.

Secondly, there is a very poor correlation between the van den Bergh and the urobilin in the urine. There is a possible fallacy in our records, as we had to trust patients to bring *their own* urine, whereas we took the blood ourselves. There is little difference between the urobilin findings in the hypochromic and orthochromic cases.

Two points emerge, namely, that there is a difference between the percentage of van den Bergh positive cases in the hypochromic series and in the orthochromic series, and in males and females, respectively, though in neither case is this difference statistically quite significant; there is, therefore, evidence of a greater imbalance between hæmolysis and bilirubin excretion or re-utilization in the orthochromic and amongst female patients. Further, it is suggested by the figures that higher bilirubin content in the females is not dependent upon the fact that more females fall into the orthochromic group as the sex difference is evident in the hypochromic series alone, and inversely, that the higher bilirubin content of the orthochromic series is not dependent on the fact that it consists mainly of females.

The percentage occurrence of hyper-bilirubinæmia in the orthochromic series is significantly greater than in the hypochromic series, and the difference is statistically significant.

Hyper-bilirubinæmia and splenic enlargement.—The correlation of the size of the spleen with the van den Bergh reaction was done in 54 cases, and the results are shown below:—

That is, there is hyper-bilirubinæmia in 50 per cent of the cases with an enlarged spleen and in only 25 per cent of those with non-palpable spleens ; this difference is suggestive but not quite significant statistically.

The splenic enlargement was proportionately distributed between the two sexes and between the hypochromic and orthochromic groups, respectively.

Hookworm infection.—Almost the whole community is infected. In this series an exact estimation of the hookworm ova was not made but in every case a stool was examined and the intensity of the infection was noted ; the findings were entered as one, two or three *plus*. It is estimated roughly that + + + indicates an infection of 10,000 eggs or more per gramme of stool, + + between 2,000 and 10,000, + between 200 and 2,000, and — less than 200. The findings in the two groups are as follows :—

Infection.	Hypochromic.	Orthochromic.	Mean of hæmoglobin content of blood in grammes.
+++ ..	12	6	4.33
++ ..	8	4	5.40
+ ..	11	4	5.64
— ..	8	1	4.59
Not examined ..	3	1	..

There is little difference between the two groups but the hookworm infection is slightly higher in the orthochromic group. There seems to be very little sign of correlation between the degree of hookworm infection and the degree of anæmia.

Eosinophil count and hookworm infection.—Fifteen cases with a three-*plus* hookworm infection had a mean eosinophil count of 15.1 per cent, whereas eight cases with ova less than 200 per gramme had a mean of 16.75 per cent. There is thus no correlation between the degree of hookworm infection and the eosinophil count.

Hyper-bilirubinæmia and hookworm infection.—Two of 18 three-*plus* hookworm-infected cases and 2 out of 8 of those with a few or no ova had a positive van den Bergh reaction, + + or + ; the proportion is higher in cases with a low hookworm infection, but the difference is not significant.

Gastric hydrochloric acid.

Gastric analysis was done in 45 cases.

Method.—The technique used was the same as that described in Part IV of this series. A gruel test-meal was given. In no instance was histamine used.

Achlorhydria.—There was achlorhydria in 5 cases (8 samples in 2 cases, 7 in one, and 5 in two); all these were females.

Two were from 31 cases of the hypochromic series.

Three were from 14 cases of the orthochromic series.

In one other case, a male in the orthochromic series, the first 7 samples contained no free acid and in the 8th there was a trace.

In two of these six cases a second gastric analysis was done. In the first, a female, the initial hæmoglobin was 2·89 grammes and the mean corpuscular hæmoglobin (MCH) 26·19 $\gamma\gamma$; after an interval of 90 days' irregular outpatient treatment the hæmoglobin had only improved to 3·3 g. and the MCH decreased to 21·86 $\gamma\gamma$, and there was still no free acid. In the second patient, also a female, a second and a third count was done after 74 days and 100 days, respectively. Some distinct improvement in the blood picture had occurred and on both occasions there was some free acid in the later gastric samples:—

Serial number of test.	Hæmoglobin in grammes.	Mean corpuscular hæmoglobin (MCH).	Maximum free acid in any sample.
1st ..	5·09	29·07	0
2nd ..	6·87	19·10	4
3rd ..	8·80	21·67	10

Fasting stomach.—Of the remaining 39 cases, from one no gastric juice could be obtained, and from 13 no acid was found in the fasting stomach; these were 5 males and 8 females, three of the latter being in the orthochromic series; the remainder showed varying amounts of free acid, up to a maximum titre of 64 c.c. N/10 NaOH.

Other samples.—Of the 40 cases in which some acid was demonstrated, 5 females and 3 males had a maximum free acidity of less than 15 c.c., that is, they showed hypo-acidity; of these 3 (one male) were from amongst the orthochromic cases. Three males and two females showed an acidity of 60 c.c. or more in one or more samples. The highest was 85 c.c. The 3 males were in the hypochromic series and both females were in the orthochromic series.

The mean of the highest observations in each analysis for the 18 males was 36·4 c.c., and for 25 females 25·7 c.c. (one male and one female are omitted as only two samples were taken in each case).

The total acidity was recorded in each case, but as it was always about 10 c.c. higher than the free acidity it is not referred to in this analysis.

The means of all the readings, including the zero readings in the achlorhydric cases, at the different time intervals, for the males, for the females, and for the orthochromic cases, have been calculated and are shown in Table II. They have been plotted on paper and are shown in Charts 4, 5, and 6.

TABLE II.

				Males.	Females.	Orthochromic cases only.
Number examined				19	26	13
Fasting juice				18·8	14·4	9
Time interval						
½ hour	..	..	..	8·5	2·6	1·2
½ "	..	..	..	14·1	6·3	8·0
¾ "	..	..	..	18·7	13·7	15·5
1 "	..	..	..	24·1	16·4	16·3
1½ hours	..	..	..	24·7	21·8	18·0
1½ "	..	..	..	27·4	26·3	23·7
1½ "	..	..	..	22·2	21·4	22·8

CHART 4.

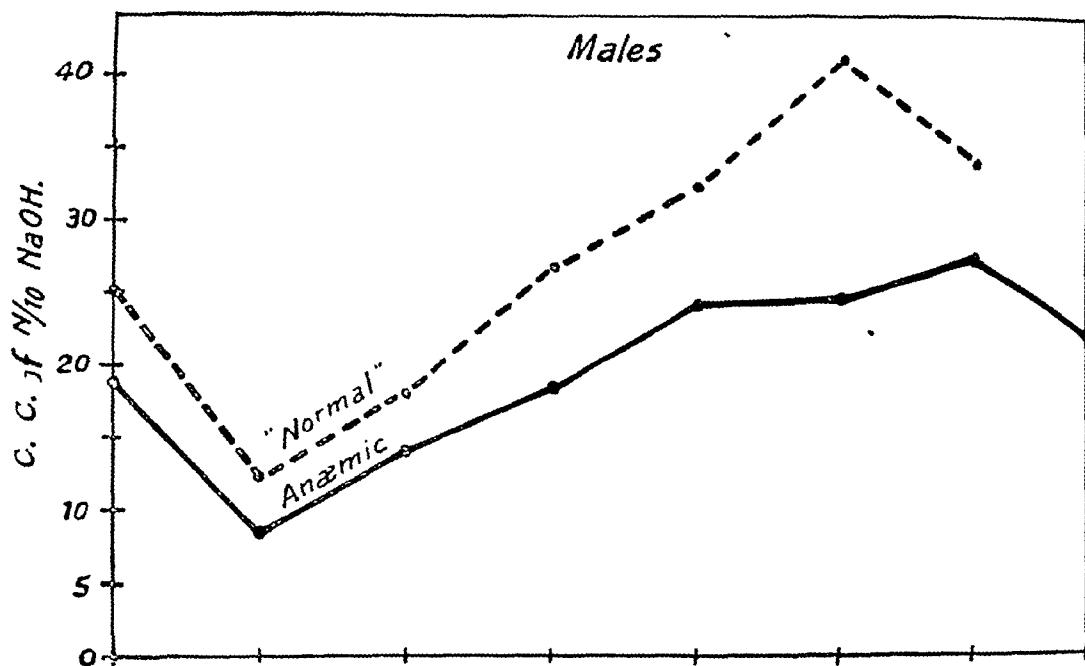
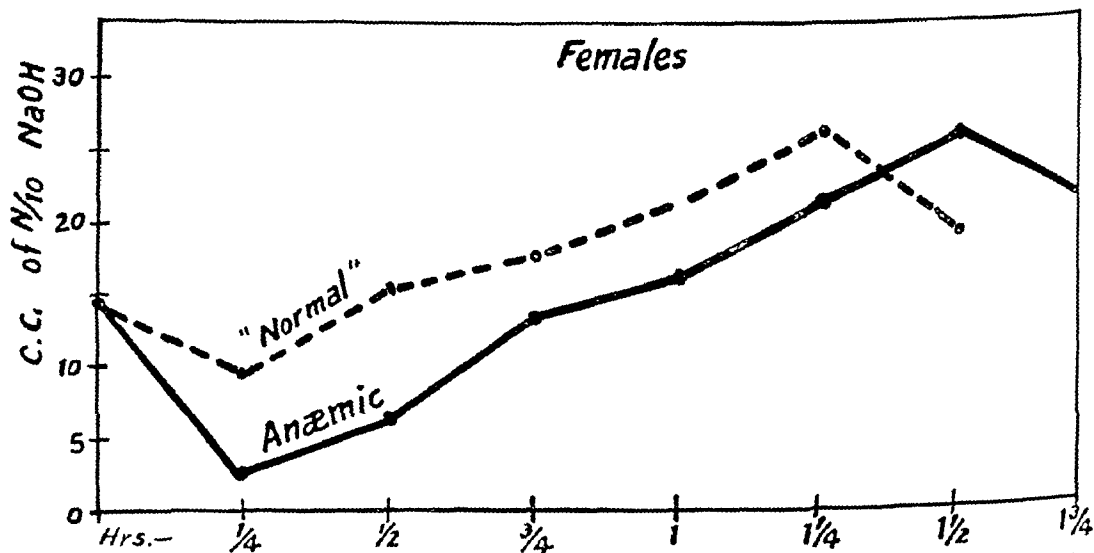


CHART 5.



CHARTS 4 and 5. Showing the mean of the acid curve in cases of anæmia contrasted with the mean curve in 'normal' coolies; the anæmic curves are based on estimations in 19 men and 26 women, respectively. A gruel test-meal was used and no histamine was given.

CHART 6.

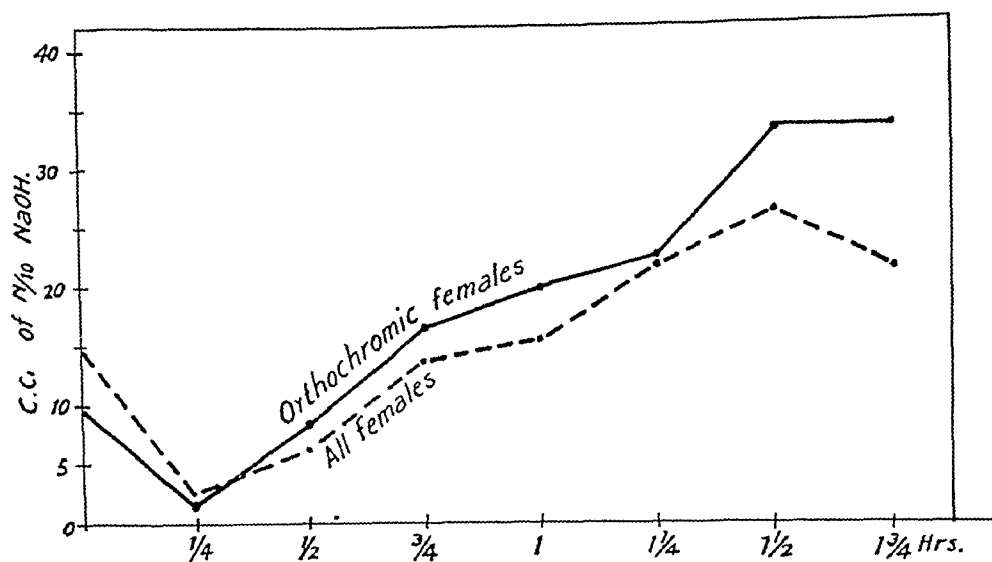


CHART 6. Showing the mean acid curve in 11 females from the orthochromic group and the mean curve in all female anemics; the curves are materially the same.

The gastric analyses are summarized in the following table :—

TABLE III.

	Hypochromic.		Orthochromic.		TOTALS.
	M.	F.	M.	F.	
Total examined ..	16	15	3	11	45
Achlorhydria throughout ..	..	2	..	3	5
No acid in fasting stomach (excluding achlorhydries).	5	5	1	3	14
Hypochlorhydria ..	2	3	1	2	8
Hyperchlorhydria ..	3	..	..	2	5

Discussion.—We will first consider the analyses as a whole. The findings do not indicate that the gastric function plays an important part in the ætiology of the anæmia in these cases. It will be seen that the proportion of achlorhydrics is scarcely greater than that in a normal series in Europe or America, when it is remembered that histamine was not given in any of these cases. However, there was only one achlorhydric in 43 'normal' persons of the same community (see Part IV). Though the mean curve is well within the normal limits of the European standard, it is slightly lower than the mean curve of 'normals' of the same population.

The mean curve of the female patients is distinctly lower than that of the males, as was the case in the normal series. Charts 4 and 5 show the curves for normals and anæmics, for men and women, respectively. In the males the curve of the anæmics is distinctly lower than that of the 'normals', but in the case of the females the curves are almost identical, except that in the anæmics it is later in reaching its maximum.

If the hypochromic group is considered separately, it will be seen that only two out of the thirty-one cases were achlorhydric; in five others the gastric secretion was below normal. There seems to be little evidence here that the anæmia could be due to failure of iron absorption through deficiency of free acid; on the other hand, it does seem probable that the slight default in gastric secretion was due to the marked anæmia.

In the orthochromic series the percentage of achlorhydria was higher—3 out of 14 cases. The man in whom there was no free acid in the first 7 samples also belonged to this group. Two other cases were hypochlorhydric, but in the remainder of the cases of this series good quantities of free acid were found, so that the mean curve is actually higher than the mean curve of the whole series at most time intervals, if allowance for sex is made (Chart 6).

Of the three achlorhydric orthochromic cases, one showed some secretion when subsequently examined, and in another there was a marked reduction in the MCH at the time of the second examination; the third case, with an MCH of 21.4 γ , would have been classified as hypochromic on European standards. Therefore none of these could be considered as a case of the pernicious anæmia type.

Results of treatment.

For this part of the Inquiry we decided that we ourselves would not undertake any treatment of these patients, nor in any way interfere with the usual procedure on the gardens in which the cases were found. However, it was the routine practice to examine every coolie on the estates at this time of year, and to give to all the anæmic patients a course of treatment for hookworm infection, followed by an iron tonic or other treatment that seemed to be indicated. Every patient in this series had one or more doses of oil of chenopodium and was given iron and ammonium citrate in pharmacopœial doses. In a few instances the patient was admitted to hospital.

Subsequent examination.—One or more subsequent examinations were made in 33 cases—12 men and 21 women; the interval between the first and last examinations varied from 45 to 120 days and averaged about 75 days. Of these only seven patients improved appreciably, that is to say, their hæmoglobin increased

by 3 grammes or more; in 6 cases there was either no improvement, or an actual decrease in hæmoglobin. The cases can be divided into the following groups according to the degree of improvement that they showed:—

TABLE IV.

Showing the results of treatment: 1st series.

Hæmoglobin increase in grammes per 100 c.c. of blood.	Hypochromic.		Orthochromic.		TOTALS.	Hospital treated.
	M.	F.	M.	F.		
3.0 or more	2	4	..	1	7	4
2.0 or more, less than 3.0	1	4	..	..	5	1
1.0 „ „ „ „ 2.0	4	3	1	..	8	..
Less than 1.0	2	1	..	4	7	1
Decrease	2	2	.	2	6	1

Discussion.—The result of treatment in these cases was very disappointing. The main reason for the failure of response to treatment was that the patients did not attend regularly for their iron-tonic treatment. This is shown by the fact that of the 7 cases in which there were more than 3 grammes improvement 4 were hospital patients, and of the 26 that showed less or no improvement only 3 were hospital patients; the difference is statistically very significant.

The improvement was distinctly better in the hypochromic cases as a whole; the one patient in the orthochromic group who improved was a hospital patient for some time; she had a good diet and possibly (we have no record of this) liver extract, as she was achlorhydric at first; if females only are considered, the difference between the improvement in the hypochromic cases and the orthochromic is more striking.

The change in the MCH between the first and the last counts was somewhat confusing; the MCH of the orthochromic cases decreased in every instance, slightly in 7 and markedly in 1, and in the hypochromic cases it increased in 19 out of 26 cases and in 6 of these sufficiently to bring it above the 20 $\gamma\gamma$ mark.

Most of the other blood examinations were repeated but the findings show so little change that the results are not worth analysing.

Conclusions based on observation made in the first series.

The only point we need consider now is whether the purely arbitrary division into hypochromic and orthochromic was justified by the other findings.

There is a preponderance of females in the orthochromic group but the difference is not significant statistically. No difference between the two groups was noted in the other blood cell counts, nor in the fragility or coagulation time.

The incidence of hyper-bilirubinæmia was definitely higher in the orthochromic series, but the difference is not statistically significant. Again there were more cases of achlorhydria amongst the orthochromic. The most striking difference was in the response to treatment, and, if females only are considered, the difference in the response between the two groups is statistically significant, but not much importance can be attached to this as the treatment was so irregular.

It, therefore, seems doubtful on the data we have analysed whether they can be considered as two separate groups, though perhaps we are justified in saying that females tend to have more heavily hæmoglobinized (and therefore probably larger) cells, and that those cases with the more heavily hæmoglobinized cells show a greater tendency to hyper-bilirubinæmia and achlorhydria, and do not respond so well to treatment by anthelmintics and iron.

THE SECOND SERIES.

The results of treatment in the first series were so disappointing that we decided to undertake another series during the following cold weather (1934-1935), to make special arrangements about the treatment of the patients, and to make more frequent examinations during the treatment. We felt that it would be advantageous to augment the series by bringing the number of anæmics examined up to at least a hundred, and to carry out more complete examinations.

In the second series, a certain amount of selection was exercised and a greater degree of anæmia was demanded. The highest hæmoglobin percentage in the series was 6.19 grammes per 100 c.c. of blood. No case was excluded on account of the special nature of his or her anæmia, other than the degree of anæmia, but, as certain dietary experiments were envisaged, notorious non-co-operators and women who had young children, to whom they might be tempted to give the extra food that was provided, were excluded.

The examinations carried out were much on the same lines as in the first series, with the few exceptions enumerated below :—

- (a) The cell volume was estimated in every case, so that the mean corpuscular volume and the mean corpuscular hæmoglobin concentration, as well as the mean corpuscular hæmoglobin could be worked out.
- (b) The hæmoglobin and the cell-volume estimations, and the red-cell and reticulocyte counts were done at frequent intervals during the course of the treatment and during 'convalescence', and in addition the white-cell count, the van den Bergh reaction, and certain other examinations were done at the end of the investigation.
- (c) A more accurate quantitative estimation of the hookworm infection was made.
- (d) The weight of the patient was taken at the beginning and the end of the investigation.
- (e) Fractional gastric analysis was attempted in every case; the first eight patients refused to swallow the gruel or vomited out the tube immediately they had swallowed it. (In this respect the average tea-garden coolie is very sheep-like; all these patients were on one garden and all the tests were done at the same time; if the first patient had kept the tube down they would probably all have kept theirs down). After this we used the alcohol 'test-meal'; this was much more popular and no difficulties arose. A fractional meal was done at a later date in the first eight cases.
- (f) For the coagulation-time test the blood was kept in warm water (37°C.).
- (g) A platelet count was not done except in a few instances and the fragility test was frequently omitted.
- (h) For the reticulocyte count a few drops of blood were taken into a small tube containing an equal quantity of cresyl blue, 1 per cent in normal saline, and a count was made on a 'wet' preparation.

The clinical material.—This was from exactly the same source as in the first series; the patients were tea-garden coolies from 5 different gardens; all but 6 were hospital patients. They presented very much the same clinical picture as those in the first series.

Age and sex.—There were originally 18 men and 24 women in the experiment; one female absconded after the first count (hæmoglobin = 2.95 g., MCH = 12.5 $\gamma\gamma$, MCV = 52.8 cu. μ , MCHC = 23.6 per cent, and a normal gastric acidity) and has been excluded from further consideration in this series.

The remainder fall into the following age-sex groups:—

Decade.	Male.	Female.
10—20 ..	6	7
20—30 ..	6	7
30—40 ..	3	7
40—50 ..	3	2

Hæmoglobin.—According to the hæmoglobin content of their blood the cases can be grouped as follows:—

Ranges in g. per 100 c.c.	Males.	Females.	TOTALS.
2.00 to 2.99 ..	2	3	5
3.00 to 3.99 ..	6	5	11
4.00 to 4.99 ..	5	8	13
5.00 to 5.99 ..	4	6	10
6.00 to 6.99 ..	1	1	2

The highest was 6.19 g., the lowest 2.40 g., and the mean of the series 4.31 g.

J, MR

The red-cell count.—According to their initial red-cell count the cases can be grouped as follows:—

Red-cell count in millions per c.mm.	Males.	Females.	TOTALS.
1.00 to 1.49 ..	1	1	2
1.50 to 1.99 ..	3	4	7
2.00 to 2.49 ..	5	7	12
2.50 to 2.99 ..	3	4	7
3.00 to 3.49 ..	4	6	10
4.00 to 4.49 ..	2	1	3

The mean corpuscular values.

The mean corpuscular hæmoglobin was calculated in all cases, and the mean corpuscular volume and the mean corpuscular hæmoglobin concentration in 38 cases: these are shown below:—

	Males.	Females.	All cases.
Mean corpuscular hæmoglobin (MCH) ..	16.17 $\gamma\gamma$	18.06 $\gamma\gamma$	17.23 $\gamma\gamma$
„ „ volume (MCV) ..	65.75 cu. μ	72.60 cu. μ	69.72 cu. μ
„ „ hæmoglobin concentration (MCHC)	25.62 per cent.	25.60 per cent.	25.61 per cent.

Thus, compared with the first series, the MCH is slightly lower, and, as before, it is higher in the females than in males. The standard error of the mean for the males is ± 0.606 and for the females ± 0.681 and the standard error of the difference is ± 0.912 ; the difference between the two means, which is 1.89 $\gamma\gamma$, is therefore just significant. All the cases but 2 are definitely hypochromic; even these 2 are below the normal MCH of 29 $\gamma\gamma$.

On the other hand, although all but 8 cases would be considered microcytic on ordinary standards, the mean of the series is not much lower than the mean of the so-called normal coolies.

The mean size of the cells in the male series is distinctly smaller than of those in the female series, but as the dispersion is considerable the difference between the means is not quite significant.

In Chart 7 the relationship of the corpuscular hæmoglobin to the corpuscular volume in 38 cases (in 3 the MCV was not recorded) is shown. This shows a fairly

CHART 7.

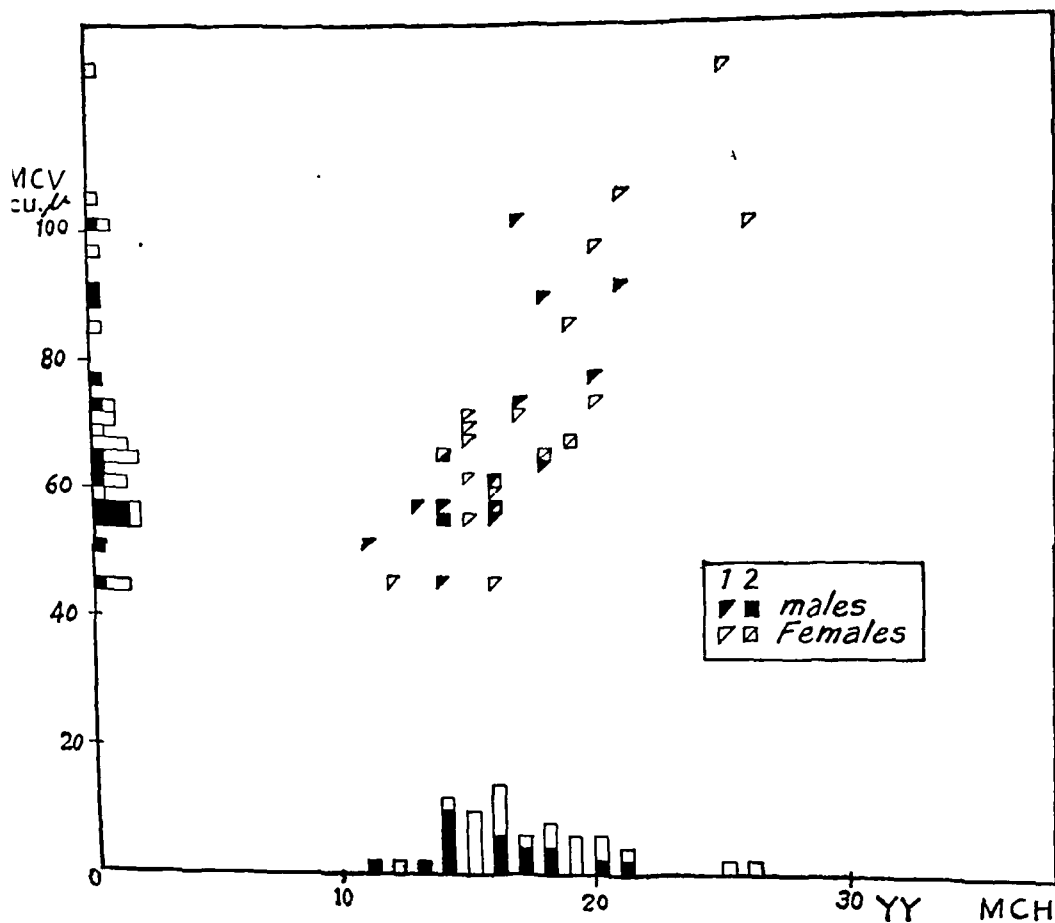


CHART 7. Showing the relationship between the mean corpuscular volume and the mean corpuscular hæmoglobin in 38 cases in the second series.

high coefficient of correlation, but it is apparent from the obtuseness of the angle which the mean line (not drawn) of these plottings makes with the base line that the mean corpuscular hæmoglobin concentration is low.

The reticulocyte count, and the total and differential white-cell counts.—These counts were done in every case; the results do not differ materially from those in the first series; they are given in tabular form below:—

TABLE V.

	Maximum.	Minimum.	Mean male.	Mean female.	Mean of series.
Reticulocyte, per cent ..	10·4	0·07	3·70	4·10	3·92
Leucocytes, in thousands per c.mm.	10·0	1·875	5·53	5·56	5·55
Lymphocytes, per cent ..	54	14	27·05	33·23	30·45
Large mononuclears, per cent.	17	1	6·61	7·19	6·92
Eosinophils, per cent ..	47	2	13·61	16·55	15·22

Few abnormal white cells were recorded in these counts so that the balance may be considered to be neutrophil polymorphonuclears; the mean of these was just under 50 per cent. In the first series the lymphocytes were about 5 per cent fewer and the polymorphonuclears correspondingly higher.

Nucleated red cells were noted in 5 cases only.

The van den Bergh reaction.

In no case was the direct van den Bergh positive.

The indirect test was done in every instance before and after treatment; the results are shown in the following table:—

TABLE VI.

Indirect van den Bergh.	BEFORE TREATMENT.		AFTER TREATMENT.			
	Males.	Females.	++	+	(+)	Negative.
Negative ..	7	10 } 26 {	..	1	..	16
(+) ..	5	4 } {	1	1	..	7
+ ..	5	6 } 15 {	..	2	1	8
++ ..	1	3 } {	1	1	..	2
			7		34	

Of the 15 van den Bergh-positive cases, 5, that is, 33 per cent, had a hookworm egg-count over 10,000; of the van den Bergh-negative cases 57 per cent

had this degree of hookworm infection. There is thus a tendency towards a negative correlation, as in the first series, but the difference is again not significant.

The association of hyper-bilirubinæmia with a relatively high MCH noticed in the first series is not apparent in this series; the mean of the MCH of the van den Bergh-positive cases is 16.84 $\gamma\gamma$ against a mean of 17.23 $\gamma\gamma$ for the whole series.

Urobilin in the urine.

The urine was tested for urobilin before treatment in 39 cases:—

	Urobilin in excess.	Normal.
Positive van den Bergh cases ..	8	5
Negative „ „ „ „ ..	5	21

Splenic enlargement.—The spleen was palpably enlarged in 13 out of 41 cases. The correlation between hyper-bilirubinæmia and enlarged spleen that was noticed in the first series is not apparent here. Of the 15 cases with a positive van den Bergh only 4 had an enlarged spleen.

Fractional gastric analyses.

The same technique was adopted except that 50 c.c. of 7 per cent alcohol was given as the 'test-meal'.

Achlorhydria.—Of the 33 cases examined before treatment, in which at least 8 samples were taken, there was only one case of achlorhydria (hæmoglobin percentage = 3.99 g.; MCH = 19.9); this was a female. In this case a second test was done and histamine given after the fifth acid-free sample; there was 20 c.c. free acid in the seventh sample, 25 c.c. in the eighth, 20 c.c. in the ninth, and 10 c.c. in the tenth.

Fasting stomach.—This was tested in all the 41 cases. The amount of juice varied from 5 c.c. to 80 c.c.; the mean was 25 c.c.; in 18 males it was 32 c.c. and in 23 females 20 c.c.

In 5 males and 10 females there was no free acid; in the rest the free acid varied from 4 c.c. to 85 c.c. N/10 HCl.

Other samples.—Post-prandial samples were only taken in 33 cases, before treatment.

Of the 32 cases in which free acid was demonstrated 3 had a maximum acidity below 15 c.c. and 3 others between 15 c.c. and 25 c.c. These six cases might be classed as hypochlorhydric in view of the higher average of the acidity in this whole series; 4 were women.

In 15 cases the maximum acidity was 60 c.c. or more; of these 10 were men. If 75 c.c. are taken as the criterion, for hyperacidity, there were 7 cases of which 5 were males with this or a higher degree of acidity.

In nine of the 16 women with free acid, the maximum acidity was reached at the third (5 cases) or fourth post-prandial sample. In 10 of the 16 males the maximum was reached at the second (7 cases) or third post-prandial sample.

The means of the samples at different time intervals are shown in Chart 8 and in the table below:—

CHART 8.

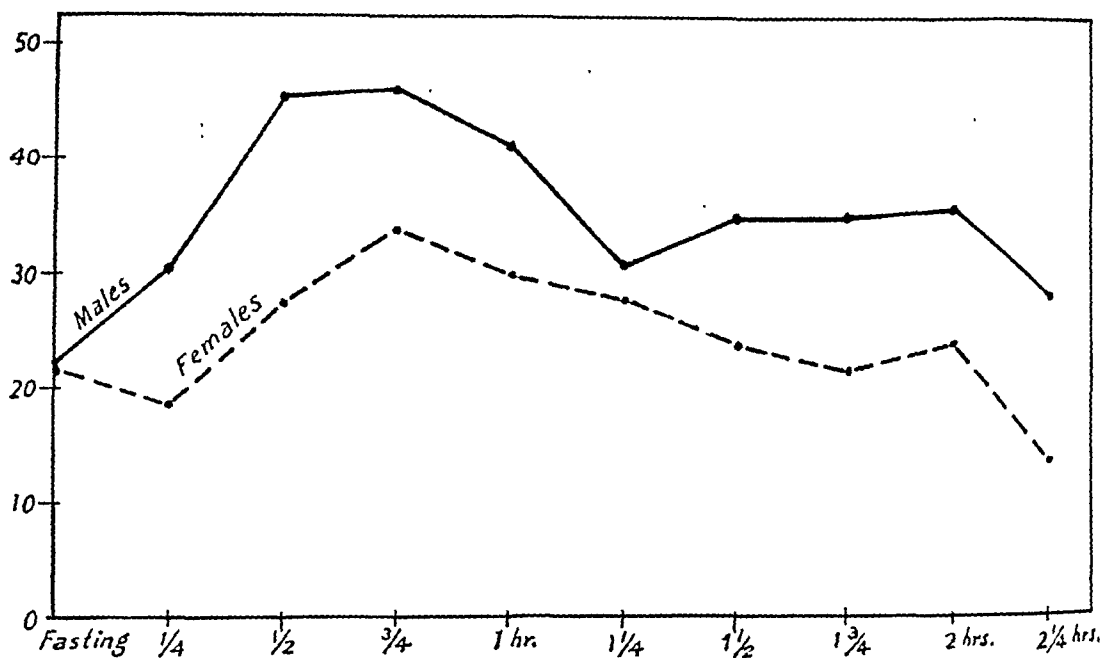


CHART 8. Showing the mean acid curve in males and females of the second series. The alcohol 'test-meal' was used; no histamine was given.

The mean of the highest observation in each test amongst the males was 57.27 c.c. and amongst the females 43.18 c.c. (the achlorhydric case included).

TABLE VII.

		Males.	Females.
Time interval \ Fasting juice		22.33	21.74
1/4 hour	..	30.33	18.82
1/2 "	..	45.64	27.88
3/4 "	..	46.28	34.12
1 "	..	41.14	30.12
1 1/4 hours	..	30.87	28.25
1 1/2 "	..	35.00	23.94
1 3/4 "	..	35.00	21.43
2 "	..	35.91 (11 cases)	23.83 (12 cases)
2 1/4 "	..	23.00 (5 ")	13.67 (6 ")
2 1/2 "	..	55.00 (2 ")	12.25 (4 ")

Re-examination after treatment.—This was not undertaken systematically. The re-examination in the achlorhydric case referred to above was after treatment. The 8 cases in which only an examination of the fasting juice was done were re-examined after treatment. One, a male patient, was achlorhydric in 5 samples, after which no juice could be obtained, 3 other cases were hypochlorhydric, and 4 had acidity within the normal range.

Discussion.—From the results that we obtained it is quite obvious that the alcohol meal stimulates gastric secretion much more than the gruel meal. The initial fall is absent in the majority of cases and the average readings are from 10 c.c. to 20 c.c. higher than in the gruel series.

In both the males and the females of the second series the mean acidity of the fasting stomach happened to be higher also; the difference is slight in the male cases but more marked in the female cases. Amongst the female cases there were actually more with complete absence of acid in the fasting stomach in the second series, but there were 4 cases with very high acid contents, 2 with a titre at 85 c.c. and 2 at 65 c.c., so that the mean is raised.

What is even more apparent than the height of the acidity is the earlier achievement of the maximum; this was delayed to about $1\frac{1}{2}$ hours in the first series, whereas amongst the male cases in the second series a number reached their maximum in half an hour, and the mean maximum was at $\frac{3}{4}$ hour in both male and female. Again, the acid curve rises earlier in the male than in the female cases, but in this series it also rises much higher.

There is evidence of a secondary rise in the curves at two hours; this was not apparent in the first series as the tests were not continued long enough.

It is unfortunate that we have no series of 'normal' observations in Indians in which this alcohol meal has been used.

Except for these few differences in the gastric analyses, which are almost certainly due to the difference in the meal, the findings support our main conclusion based on the first series, namely, that deficiency in acid secretion can play no part on the ætiology of the anæmia in these cases.

Hookworm infection.

The stools were examined in every case for helminthic ova at the beginning of the experiment and again after treatment had been given. In most cases intermediate examinations were done but we are not recording these.

Technique.—The egg-counts were made by placing a given bulk (3 g.) of stool, measured by fluid displacement, in a test-tube, making up to 90 c.c. with N/10 caustic soda, transferring the whole to a bottle, and mixing thoroughly with the help of glass beads; 0.15 c.c. of diluted stool is placed on a glass slide, covered with a large coverslip, and all the ova on the slide counted. The dilution is arranged so that the number of eggs on the slide multiplied by 200 gives the total number of eggs per gramme of stool.

All methods of egg-counting are essentially rough, and give little accurate information as to the load of infection in an individual case, but this method probably gives a fairly accurate indication of the degree of infection in a group of cases.

Hookworm, ascaris and whip-worm ova were found in practically every case. The ascaris infection was often very high, but as there is little reason to associate this infection with anæmia we shall only consider the hookworm infection here.

The results of the initial hookworm egg-counts are given below:—

Load. Eggs per gramme.	Number of cases.
(a) Over 10,000	21
(b) Less than 10,000 but over 5,000 ..	10
(c) Less than 5,000 but over 2,000 ..	6
(d) Less than 2,000 but 200 or over ..	4
(e) Less than 200	0

These figures suggest that the infection was rather heavier in this than in the first series, but it must be remembered that in all the cases of the first series accurate counts were not made.

These egg-counts indicate that the hookworm infection rate amongst the anæmic coolies is very high, in fact 100 per cent. They also show that the load of worms is considerable in the majority. As in the first series, there is no correlation between the degree of anæmia and the degree of the hookworm infection. The 21 cases in which there are 10,000 or more eggs per gramme of stool are distributed as follows:—

TABLE VIII.

Initial hæmoglobin in g. per 100 c.c.	Distribution of cases.	Distribution of heavily infected cases, 10,000 eggs or over.
Less than 3 g. ..	5	2
Over 3 g. but less than 4 g. ..	11	7
„ 4 g. „ „ „ 5 g. ..	13	6
„ 5 g. „ „ „ 6 g. ..	10	6
„ 6 g.	2	..

Control counts.—We counted the eggs in over 500 random samples of stools from the general coolie population on the same gardens, but the figures had to be discarded as the technique adopted was open to criticism. From stool examination in a smaller number of 'normal' coolies (42) we found that the incidence of infection in the general coolie population was almost 100 per cent, but that only about 14 per cent had counts above 10,000, against 51 per cent in the present series.

The higher incidence of heavy infections amongst the anæmic coolies does suggest that hookworm infection plays some part in the ætiology of this anæmia, though the absence of any relationship between the degree of the anæmia and the number of ova in the stools does not support this suggestion.

Coagulation time.—This was done in only 25 cases in the second series. The mean coagulation time was 2.88 seconds; it was about the same in males and females. The marked difference in these results and those of the first series is due to a difference in technique. It is evident that there is no marked departure from the normal.

The results of treatment.

In the second series of cases we arranged with the doctors in charge of the cases to undertake the anti-anæmic treatment ourselves. The course of treatment

CHART 9.

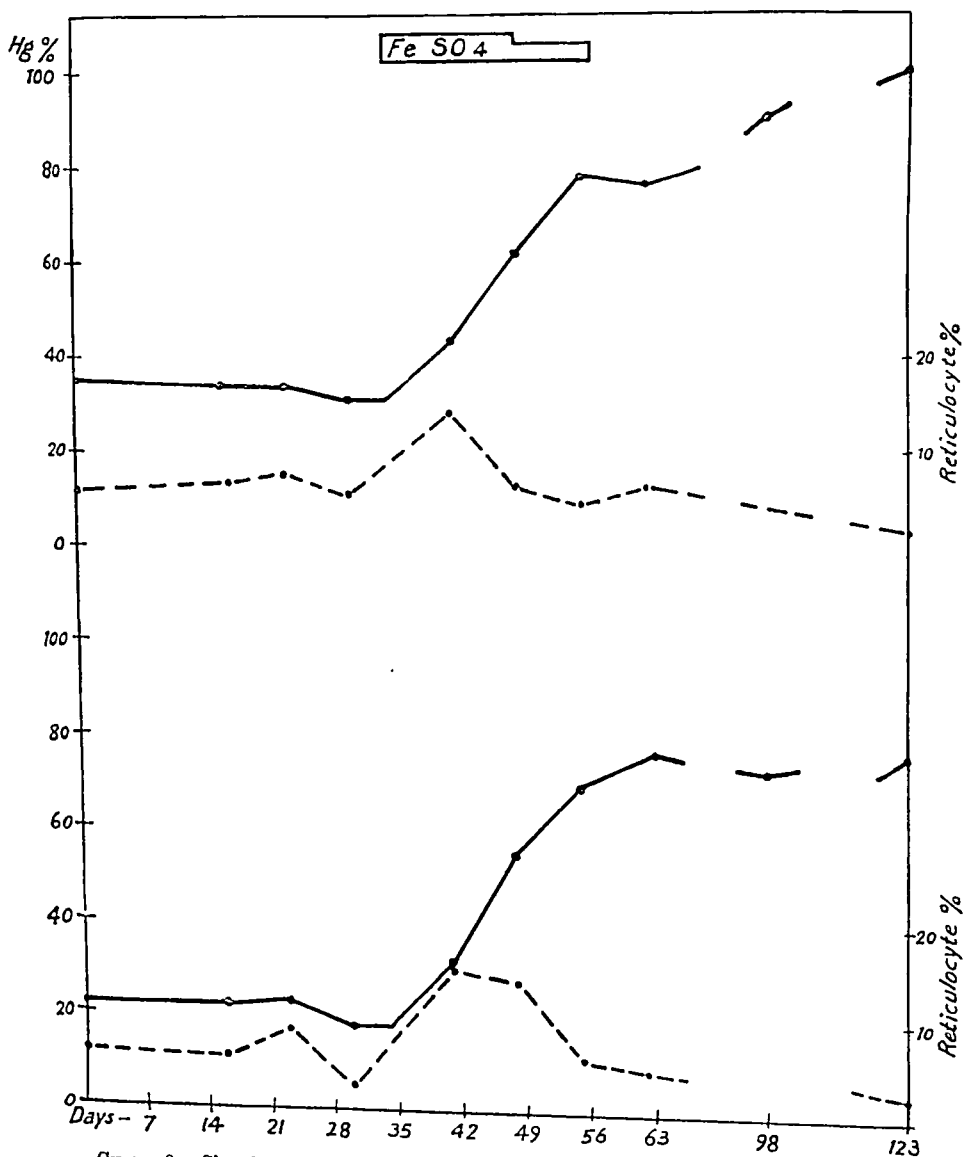


CHART 9. Showing the response to iron therapy in 2 patients who were kept on a good diet of high caloric value for one month before and during treatment. Anthelmintic treatment was given after the iron administration had been discontinued. (— = hæmoglobin; --- = reticulocytes).

and the mode of procedure were varied from garden to garden ; the different results obtained will be discussed in detail below. In every case the ' specific ' treatment

CHART 10.

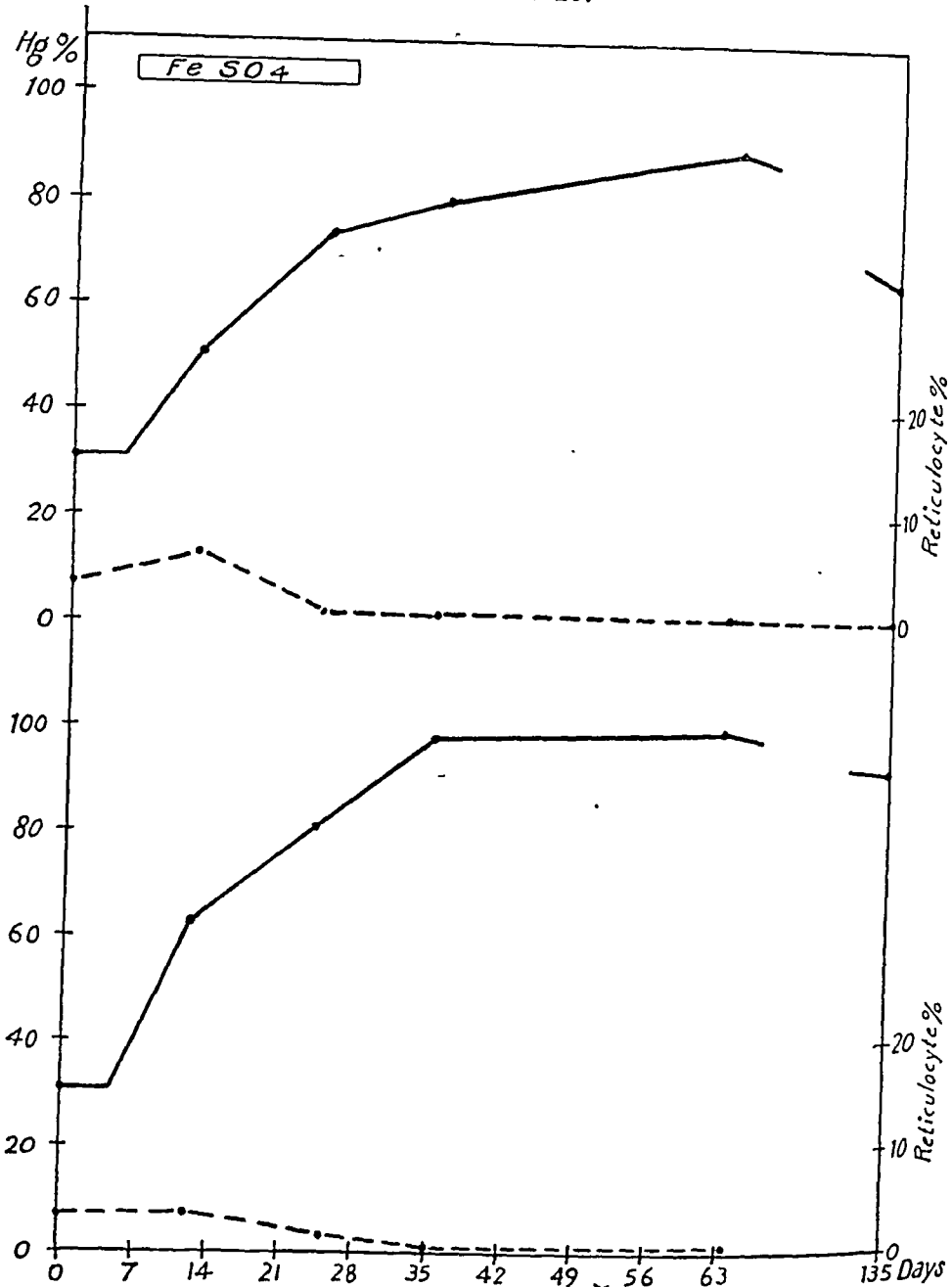


CHART 10. Showing the response to iron therapy in patients who were on a normal diet throughout. Anthelmintic treatment was given after the iron treatment. (— = hæmoglobin ; - - - = reticulocytes).

given was ferrous sulphate. We used the ferrous sulphate in two forms, Glaxo tablets which contain 3 grains of the iron salt, and a trace of copper and

manganese, and tablets made for us by Messrs. Smith, Stanistreet and Co.; these latter contained $5\frac{1}{2}$ grains of the iron salt, also with a trace of copper and manganese. From considerable experience with these two preparations we have every reason to believe that the ferrous sulphate contained in either of these tablets is fully active. All but 6 patients were in hospital throughout the course of treatment.

We will first consider the results of treatment as a whole.

Thirty-nine out of 41 patients responded to treatment in varying degrees; the poorest response in these cases was in a patient in whom the hæmoglobin increased from 3.07 g. to 4.40 g., that is by 1.33 g. (or 9.5 per cent), and the best in a patient in whom the improvement was from 3.99 g. to 15.81 g., that is 11.82 g. (or 86 per cent). The period of observation was about four months in all but one group of 7 cases in which it was three months, and the improvement referred to here is the difference between the first and the final estimations of hæmoglobin. The characteristic improvement following iron treatment in individual cases is shown in Charts 9 and 10.

According to the degree of improvement the cases can be grouped as follows:—

TABLE IX.

Showing distribution of cases according to degrees of improvement during treatment.

			Male.	Female.	TOTALS.	Initial egg- count over 10,000.	Final egg- count over 5,000.	Van den Bergh- positive cases.
Decrease of less than 1 g.	..	..	..	1	1	1	1	..
Improved less than 1 g.	..	..	..	1	1	1	1	..
Improved 1 g. but less than 2 g.	..	..	1	..	1	1	..	..
" 2 " " " "	..	..	2	..	2	2	2	1
" 3 " " " "	..	..	1	..	1	1	1	..
" 4 " " " "	..	..	2	1	3	..	..	..
" 5 " " " "	..	..	3	4	7	5	2	1
" 6 " " " "	..	..	1	5	6	3	..	3
" 7 " " " "	..	..	2	6	8	2	1	6
" 8 " " " "	..	..	1	5	7	3	4	1
" 9 " " " "	..	..	4	..	3	1	1	2
" 10 " " " "	..	..	..	..	..	..	..	..
" 11 " " " "	..	..	1	..	1	1	..	1
TOTALS ..			18	23	41	21	13	15

Calculated from this table, the mean improvement in the whole series is 6.38 g., in the males 6.39 g., and in the females 6.37 g. The four best results

obtained were in males and the two cases that failed to respond were females, but on the whole the response to treatment in the two sexes was equal.

According to the final level of hæmoglobin reached the cases can be grouped as follows :—

TABLE X.

Showing distribution of cases according to final hæmoglobin level.

			Males.	Females.	TOTALS.
15 g. but less than 16 g.	..	..	1	..	1
14 „ „ „ „ 15 „	..	..	2	..	2
13 „ „ „ „ 14 „	..	..	3	2	5
12 „ „ „ „ 13 „	..	..	3	4	7
11 „ „ „ „ 12 „	..	..	..	7	7
10 „ „ „ „ 11 „	..	..	1	3	4
9 „ „ „ „ 10 „	..	..	..	4	4
8 „ „ „ „ 9 „	..	..	4	1	5
7 „ „ „ „ 8 „	..	..	2†	..	2
6 „ „ „ „ 7 „	..	..	1	..	1
4 „ „ „ „ 5 „	..	..	1*	2*	3

Note.—For reasons given later in the paper, four cases have been classed as ‘unsatisfactory’.

* Cases classed as ‘unsatisfactory’.

† One of these was ‘unsatisfactory’.

If the 4 ‘unsatisfactory’ cases are excluded, the mean of the whole series, calculated from the above table, is 11·26 g., of the males 11·32 g. and of the females 11·21 g.

In the original hæmoglobin estimations in this series the tendency of males to have a higher hæmoglobin percentage is shown only in the greater number of females in the series. In the above table it will be seen that the male cases fall into two groups: 12 grammes and over, and 8 grammes and under; 2 of the lower group are the cases referred to as ‘unsatisfactory’, and 5 out of 6 of the remainder come from the garden (Kharikatea, *v.i.*) on which the results of treatment on the whole were poor. The inclusion of this group, in which there is reason to suppose that the effect of treatment was not maximal through extraneous causes, in the male series has marked the re-establishment of male superiority in hæmoglobin percentage. The mean in the female series, excluding the two ‘unsatisfactory’ cases, is more than a gramme higher than the mean of ‘normal’ coolie female population (*vide* Part III).

The improvement in relationship to the initial hæmoglobin level is shown in the table below :—

TABLE XI.

Group.	Number in group.	Mean of initial hæmoglobin.	Mean of final hæmoglobin.	Difference.
2.00 to 2.99	5	2.64	9.27	6.63
3.00 to 3.99	11 (9)	3.41 (3.47)	9.95 (10.84)	6.54 (7.37)
4.00 to 4.99	13 (12)	4.57 (4.55)	11.35 (11.90)	6.78 (7.35)
5.00 to 5.99	10 (9)	5.44 (5.48)	10.80 (11.55)	5.36 (6.07)
6.00 to 6.99	2	6.15	12.78	6.63

The total mean improvement is 6.35 grammes.

The figures in brackets are calculated after omitting 4 'unsatisfactory' cases.

The first impression given by the above figures is that the degree of improvement is fairly constant, and that the final level of hæmoglobin reached is dependent on the initial level.

As this is an important point we decided to calculate from the individual readings the coefficient of correlation between the initial hæmoglobin and the degree of improvement, and between the initial hæmoglobin and the final hæmoglobin level. We, however, considered that the cases in the unsatisfactory group (Kharikatea—outdoor) should also be excluded and the coefficients of correlation (r) have been calculated in the remaining 33 cases; they are, respectively, $r = -0.301$ and $r' = +0.460$; that is, there is a negative correlation between the initial level of hæmoglobin and the improvement during treatment, and there is a positive correlation between the initial and final hæmoglobin levels, the latter correlation being the higher. This means that there is a greater tendency for the hæmoglobin to increase by a fixed amount, but that there is also some limiting influence which comes into force when the higher levels of hæmoglobin are reached, so that those bloods with a lower initial hæmoglobin level have greater scope for increase.

The immediate effect of the iron treatment and the maintenance of improvement.—

Three or four examinations were made during the course of the iron treatment, and in every case a complete examination was made about one month (from 28 to 32 days) from the beginning of the iron course. A final examination, of which we have already analysed and shown the results, was made two or three months later. Between the count at the end of the iron treatment and the final count, the 4 'unsatisfactory' cases being excluded, 20 showed a further increase in hæmoglobin, 9 a decrease, and 6 no change; in 2 cases the coolies were not available when the other final counts were done. The mean of the further increase in these 35 cases was 0.44 g.

In 22 cases an additional count was done one month after the conclusion of the iron treatment; of these 19 showed an increase, 1 no change and 2 a slight decrease. The mean increase in these 22 cases was 0.91 g. At a final

examination in 8 of these cases, one month later, there had been a further increase in 6, in 1 there was no change, and there was a slight fall in the other; in the 8 cases there was a mean increase of 0.72 g. At the final examination in the other 14 of these cases, two months later, only 2 showed a further increase, 2 showed no change, and 10 showed a decrease; the mean difference between the final count and the last count but one in the series was a 0.54 g. decrease. Thus, in this last group of cases, the hæmoglobin appeared to rise for a month to two months after the conclusion of treatment and then it started to fall again.

The red-cell count.—According to their final red-cell count the cases can be grouped as follows:—

TABLE XII.

Red-cell count in millions per c.mm.	Males.	Females.	TOTALS.
1.50 to 1.99 ..	1*	1*	2
2.00 to 2.49 ..	..	1*	1
3.00 to 3.49 ..	1*	1	2
3.50 to 3.99 ..	5	3	8
4.00 to 4.49 ..	2	1	3
4.50 to 4.99 ..	1	5	6
5.00 to 5.49 ..	3	6	9
5.50 to 5.99 ..	4	5	9
6.00 to 6.49 ..	1	..	1

* These are the 4 cases classed as 'unsatisfactory'.

If the 4 'unsatisfactory' cases be excluded, the mean of the whole series is 4.93 millions, of the males 4.97 millions and of the females 4.89 millions.

The mean corpuscular values.—The mean corpuscular values at the end of treatment are shown in the table below:—

TABLE XIII.

	Males.	Females.	TOTALS.
Mean corpuscular hæmoglobin (MCH) in $\gamma\gamma$..	23.00	23.20	23.11
Mean corpuscular volume (MCV) in cubic μ ..	74.72	74.24	74.45
Mean corpuscular hæmoglobin concentration (MCHC) per cent.	31.78	31.85	31.82

These figures indicate a decided movement in all the values towards the normal figure. The sex difference seems to have disappeared entirely. Only in the MCHC do they, however, approximate to the normal. A distribution curve of the MCHC values shows a marked concentration round the 31—33 neighbourhood with only isolated incidences below this figure; of the 4 cases below 26 per cent 3 are amongst the 4 'unsatisfactory' cases referred to above and in the fourth there was possibly an error in technique, as in two previous post-treatment examinations the MCHC had been 32 and 29, respectively.

There is thus a deficiency of a little less than 20 per cent in both the hæmoglobin content and the size of the cells, but the saturation of the cells has returned to a figure well within the normal range.

Reticulocytes.—As the gardens were some distance from the laboratory it was not possible to do daily counts, so that in the majority of cases the peak of the reticulocyte rise will have been missed. The examinations were done in different cases from the eighth to the eleventh day, but the majority were done on the ninth or tenth day of iron treatment.

The findings can be tabulated as follows :—

TABLE XIV.

Showing distribution of cases according to their maximum reticulocyte response.

Maximum reticulocyte response.		Males.	Females.	TOTALS.	Mean initial hæmoglobin level of group to one place of decimals, in grammes.
2 per cent but less than 3 per cent ..	..	..	1	1	5.5
3 " " " " " 4 " " "	1	1	1	1	6.2
4 " " " " " 5 " " "	2	3	5	5	5.3
5 " " " " " 6 " " "	2	2	4	4	4.0
6 " " " " " 7 " " "	2	2	4	4	3.8
7 " " " " " 8 " " "	4	1	5	5	3.8
8 " " " " " 9 " " "	..	4	4	4	3.8
9 " " " " " 10 " " "	1	..	1	1	2.4
10 " " " " " 11 " " "	1	1	2	2	4.0
11 " " " " " 12 " " "	1	1	2	2	3.9
12 " " " " " 13 " " "	1	1	2	2	3.9
13 " " " " " 14 " " "	..	2	2	2	4.0
14 " " " " " 15 " " "	..	1	1	1	3.4
16 " " " " " 17 " " "	..	2	2	2	3.2
22 " " " " " 23 " " "	..	..	1	1	4.7

The 4 'unsatisfactory' cases have been excluded from the table; none of them showed a response higher than 6 per cent.

The reticulocyte response was lower than one would have expected, but this must be due mainly to the fact that in most cases the peak was missed. There is little indication of a relationship between the reticulocyte rise and the initial hæmoglobin level, though the 7 cases showing the least response were certainly amongst the cases with the highest initial hæmoglobin. (Further reference will be made to the reticulocyte responses on the different gardens.)

In 26 cases the reticulocytes fell to below 1 per cent at the final count. This is lower than the average count for the 'normal' coolie of this population. The remaining 15 include the 4 'unsatisfactory' cases. In 3 cases only was the final count about 2.6 per cent and 2 of these were 'unsatisfactory' cases.

The mean initial reticulocyte count in the whole series was about 4 per cent with a maximum of about 10 per cent. This degree of reticulocytosis, whilst being a little higher than that usually associated with hookworm infection, is not of the degree that one gets in hæmolytic anæmias and can probably be explained on the grounds of the stimulation of anoxæmia. The fact that the count in the vast majority fell below the level usually found in the 'normal' coolie population suggests that the hæmopoietic system in these cured anæmics was in a more balanced state of equilibrium than it is in the so-called 'normal' coolie.

Van den Bergh reaction—The results after treatment are shown in Table VI. The difference between the percentage of positive findings before treatment is just on the significance level, and the latter are not significantly different from the positive results in 'normal' coolies, shown in Table I.

The effects of anthelmintic treatment.

The treatment given was that which was usually employed on the garden in question. The drugs used were oil of chenopodium and tetrachlorethylene; from one to three treatments with one of these drugs or with a combination of the two were given. The efficacy of the anthelmintic treatment given is not a point we propose to discuss here.

The load of infection after treatment is shown in the table below :—

TABLE XV.

Group before treatment Number in group Group into which they fall after treatment			(a)	(b)	(c)	(d)	Grouping after treatment.
			21	10	6	4	
(a)							
10,000 +	..	..	8	1	..	1	10
(b)							
5,000 +	..	..	1	..	1	1	3
(c)							
2,000 +	..	..	4	4	2	1	11
(d)							
200 +	..	..	8	3	1	1	13
(e)							
200 -	..	..	..	2	1	..	3
Not done	..	..	..	..	1	..	1

Hookworm infection and progress under treatment.

In column 5 of Table IX the relationship of the initial hookworm egg-count to the progress under treatment is shown.

The mean of the hæmoglobin improvement in the series in which there were more than 10,000 eggs per gramme of stool was 5.74 g. and in that in which there was less, 7.10 g.; the standard errors of the means in the two series are respectively ± 0.6585 and ± 0.3528 and of the difference ± 0.7470 . The difference, 1.36 g., is thus just short of the significance level.

But, if we take an arbitrary division at 4 g. improvement, it will be seen that every patient who failed to improve by 4 g. had an initial degree of hookworm infection represented by at least 10,000 eggs per g. The difference in the percentage of cases that show improvement above this figure (4 g.) in the two groups, above and below 10,000 eggs, is definitely significant.

In considering the egg-counts after treatment, in order to include a slightly larger number of cases we have taken 5,000 eggs as the point of division. The distribution of these cases is shown in column 6 of Table IX.

The mean of the hæmoglobin improvement in the heavily infected group is 5.50 g. and in the rest 6.79 g.; this difference is again not quite significant. It will be seen, however, that 5 out of 6 cases that showed an improvement of less than 4 grammes had 5,000 or more (10,000 in four cases) eggs per gramme of stool at the end of the period of observation. Again, the difference in the percentage of those that showed more than 4 g. of improvement in the two post-treatment hookworm infection groups is definitely significant.

Thus, there is some evidence that the initial load of hookworms has some influence on the progress of the patient under iron treatment, though many of the patients with a heavy load progressed very favourably. There is also evidence that getting rid of the infection has some effect on the progress; although in 6 cases in which a heavy infection persisted the increase was more than 7 g. (51 per cent), of the cases in which there was little progress nearly all were cases in which the infection had persisted.

Other possible factors in prognosis.

The mean improvement in 11 cases that showed achlorhydria or hypochlorhydria was 6.91 g.; this figure is slightly higher than the mean of the whole series.

In the 15 cases in which the van den Bergh indirect test was positive before treatment, the mean improvement was 7.43 g. and the mean in 26 negative cases was 5.73; in 7 cases in which the van den Bergh was positive at the end of treatment the mean improvement was 6.18 g.

In the 13 cases with an enlarged spleen the mean improvement was 5.64 g.

Of these the only significant departure from the mean of the whole series is in the case of the pre-treatment van den Bergh-positive cases.

PROCEDURE ON DIFFERENT GARDENS.

On each of the five different gardens on which we undertook the treatment of these cases, we adopted different procedures. Our original intention was to have six groups, each consisting of 8 cases, but we had to modify our plans on account of shortage of suitable cases. In three groups we had only 6 cases.

The experiment was commenced with 42 cases, but, as already noted, 1 coolie absconded after the first examination and her data have not been included in any of the tables or other calculations; 2 others were not available for the final examination, but the rest were kept under observation for the whole period of 3 or 4 months.

Excluded cases.—As we were making a comparison of the different procedures that we adopted and as the numbers in each group were small, we felt that we ought to exclude certain cases in which the response to treatment was influenced by exceptional circumstances. The reasons for the exclusion in each of the 4 cases are given below:—

- (a) Male, aged 24, Nagadhohie garden. He was addicted to alcohol and did not give up his habits during treatment; he had achlorhydria. His progress under treatment was not entirely unsatisfactory, as he showed a slight reticulocyte response (7.9 per cent) and his hæmoglobin improved by 4.00 g. in the first month of iron treatment. He was a normocytic case but hypochromic and obviously a case of iron-deficiency anæmia.
- (b) Female, aged 35, Kharikatea garden, hospital case. She was a microcytic-hypochromic case. She showed no reticulocyte response and improved only by about a gramme during treatment, but by the final examination her hæmoglobin was back to its original figure. She had a very heavy hookworm infection; anthelmintic treatment produced no improvement in this. This was obviously not an uncomplicated iron-deficiency case.
- (c) Male, aged 40, Kharikatea garden, out-patient. He was an alcoholic and a very troublesome coolie. His habits could not be controlled as he was an out-patient. There seems some doubt if he retained the iron tablets. He showed no reticulocyte response and his total improvement was just over a gramme.
- (d) Female, aged 30, Kharikatea garden, out-patient. A microcytic-hypochromic case, she responded to iron treatment at first, showing an increase of 3.64 g. at the end of a month, but without reticulocyte response. Within a week the hæmoglobin fell by nearly a gramme and after another 2½ months it had fallen to 0.9 g. below its original level. She had a very heavy hookworm infection that was unaffected by treatment. She was undoubtedly a case of iron-deficiency anæmia, but there must have been some other factor causing the rapid recurrence. It is doubtful if the hookworm infection alone could account for this.

Nagadhohie (Chart 11).

There were 6 females and 1 male in this series, 1 male having been excluded. They were all hospital patients. The mean hæmoglobin of the group was 4.4 g.

Dietary treatment.—The patients were kept on a special diet consisting of milk, eggs, chicken or mutton, sugar, and oranges daily, with fish occasionally, in addition to their ordinary basic diet of rice, dāl, vegetables, and mustard oil (*see Appendix*). The total caloric value of this diet was about 3,500. Of these about four hundred were from protein (half of animal origin), eight hundred from fat (one-third of animal origin), and the rest from carbohydrate. Cod-liver oil and malt was given, a teaspoonful a day during part of the time.

NAGADHOLIE.

Per cent reticulocytes - - - - -
 Per cent Hb. (Heluge) ————
 Millions R.B.C. ————

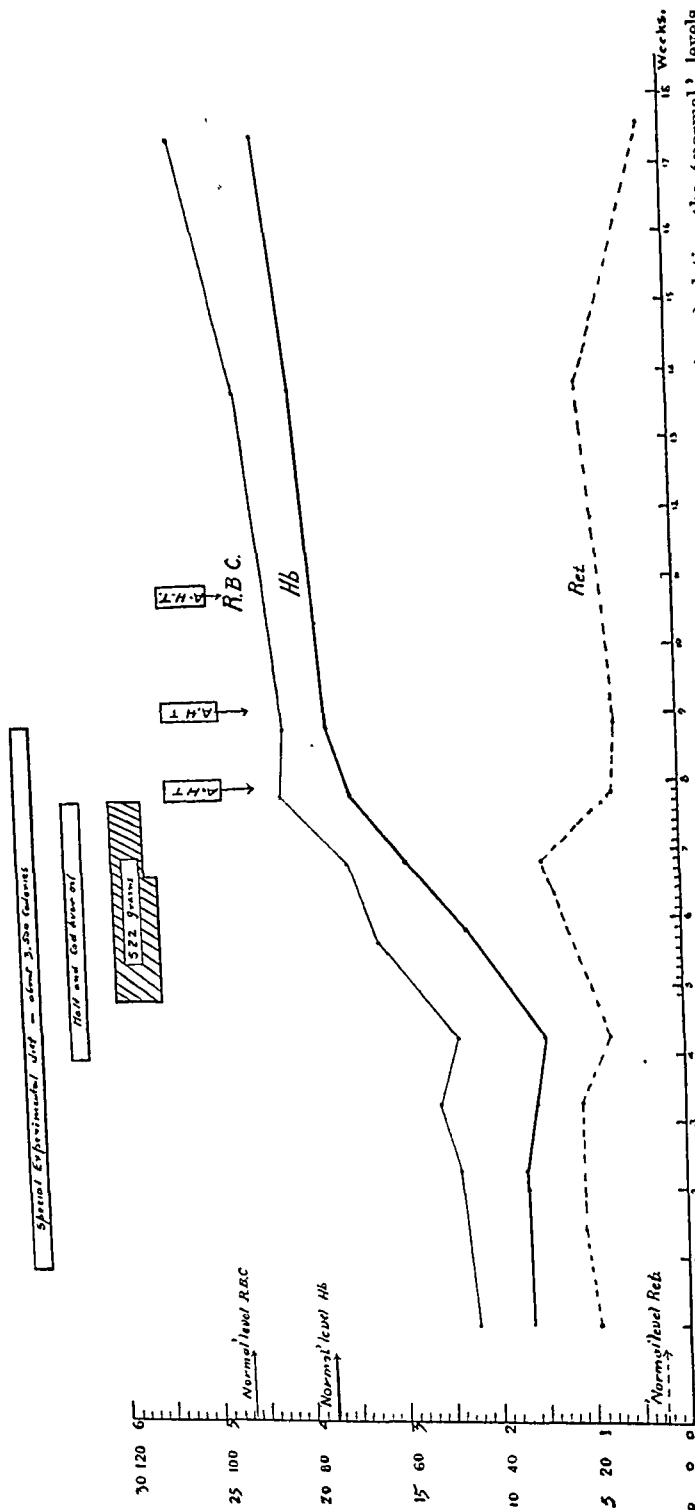


CHART 11. Showing composite curves based on the findings in 7 cases, 1 male and 6 females; in calculating the 'normal' levels in this and the subsequent figures the sex composition of the group has been taken into account.

For the first 28 days this dietary was given; cod-liver oil was added, during the last week, but no iron was given; during this period three blood counts (subsequent to the initial count) were done in each case.

Response.—No improvement occurred in the blood picture in any case and the mean hæmoglobin had fallen from 4.4 g. to 4.0 g. at the end of the month. The weight remained constant or there was a slight decrease; however the general condition of the patients was much better and some had lost their puffiness.

Iron treatment.—The diet was continued for another month and two teaspoonfuls of cod-liver oil were given twice daily; in addition 27 grains of ferrous sulphate was given daily for the first fortnight and 18 grains daily for about another week. The total amount actually given to each patient was 522 grains.

Response.—In every case the response to treatment was immediate and the mean hæmoglobin in the 7 cases was 10.1 g. at the end of 28 days from the commencement of the iron treatment.

Hookworm treatment.—The special diet was discontinued and three doses of oil of chenopodium were given at 10-day intervals.

Progress.—A count was done after an interval of one month from the last count and the mean hæmoglobin was found to have increased to 10.9 g. At a final count one month later the mean hæmoglobin percentage was 11.7 g. There was a mean increase of four pounds in the weight of the patients during the four months of observation.

Murmur (Chart 12).

In this series there were 3 males and 5 females; they were all treated as hospital patients. The initial mean hæmoglobin level was 3.9 g.

Diet.—They were placed on a diet very similar to the one in the Nagadholie group. The caloric value of the diet was also about 3,500, and fat-protein-carbohydrate proportions were the same as in the previous group. Two teaspoonfuls of malt and cod-liver oil were given twice a day. This was given for 38 days, and then the patients were given normal hospital diet.

Iron.—From the ninth day of observation a dose of 6 grains of ferrous sulphate was given three times a day for 30 days, a total amount of 540 grains.

Response.—In every case the hæmoglobin increase was immediate, but in the majority there was no marked reticulocyte increase on the 17th day (eighth day of iron treatment) when the first post-treatment count was done.

On the tenth day (first day of iron) the hæmoglobin had undergone no appreciable change (the chart shows a slight rise, which is a drawing error). On the thirty-eighth day the mean hæmoglobin had risen to 10.3 g.

Hookworm treatment.—On the thirty-ninth and the fifty-third days each patient was given anthelmintic treatment, 1 c.c. of oil of chenopodium and 3 c.c. of tetrachlorethylene.

Progress.—On the seventy-fifth day, 66 days after the commencement of the iron treatment, the mean hæmoglobin had risen further to 11.8 g. but at the final count two and a half months later it had fallen to 11.3 g.

CHART 12.

MURMURIA.

Per cent reticulocytes
 Per cent Hb. (Hellige)
 Millions R.B.C.

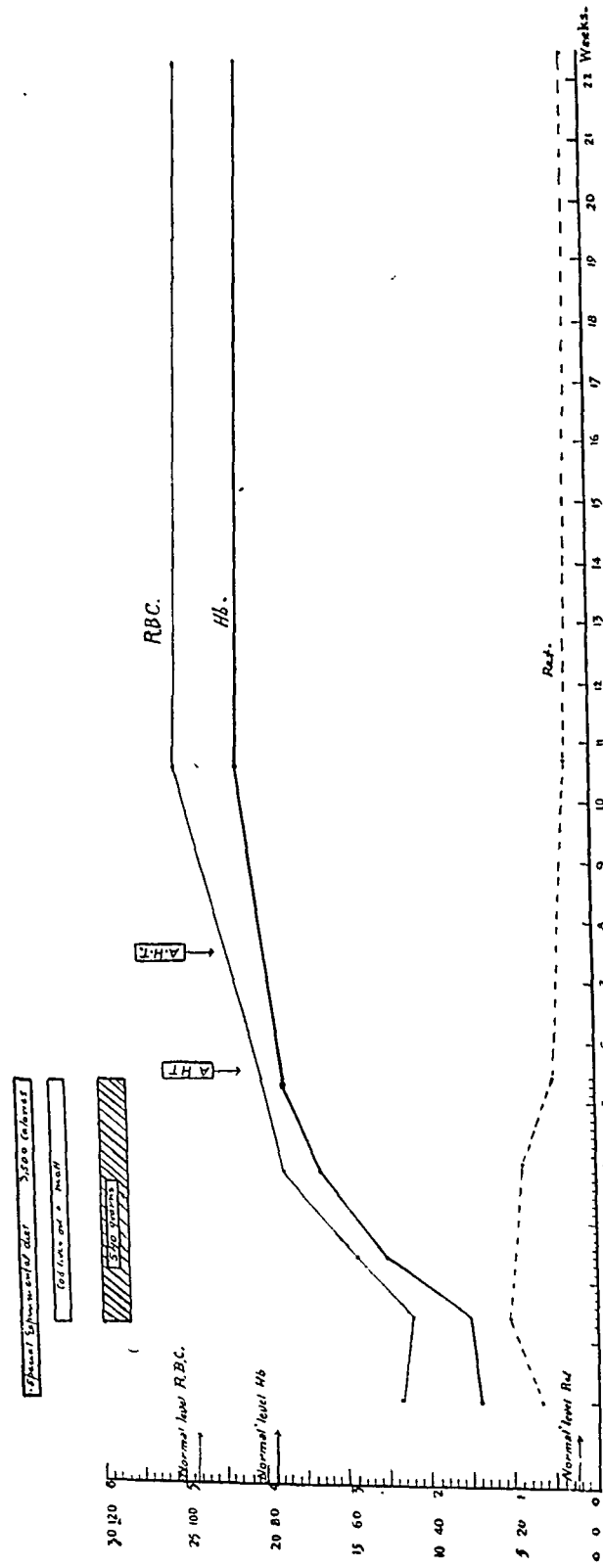


CHART 12. Showing composite curves based on the findings in 8 cases, 3 males and 5 females.

Weight.—At the end of the iron treatment 5 had gained in weight and 3 had lost weight: 1 patient lost 7 lb. The mean increase of the group was $\frac{3}{4}$ lb.

At the end of the period of observation all but 2 had gained in weight and the mean increase of the group was 3.375 lb.

Sycolla (Chart 13).

There were 4 females and 2 males in this group; they were all hospital patients. The mean hæmoglobin in the series was 5.1 g.

Diet.—No special diet was given to these patients; they all had the usual hospital diet which contains a relatively low proportion of protein and has a caloric value of about 2,200. No cod-liver oil was given.

Iron.—They were given 18 grains of ferrous sulphate in three daily doses for 20 days, a total of 360 grains.

Response.—In every case there was a marked rise in the hæmoglobin level, but in only 1 case was a reticulocyte rise noted on the eighth day of iron treatment. On the thirtieth day of treatment the mean hæmoglobin in the 6 cases was 11.5 g.

Hookworm treatment.—Anthelmintic treatment was given immediately the iron treatment had been stopped. The same dose of oil of chenopodium and tetrachlorethylene was given, but only on one occasion.

Progress.—At the end of another month the mean hæmoglobin level had risen to 11.7 g. but two and a half months later it had fallen to 11.0 g.

Weight.—Three gained in weight at the end of the first month and 3 remained stationary—mean gain 3.17 lb. At end of observation all but 1 had gained and the mean gain was 7.17 lb.

Katunibari (Chart 14).

There were 4 females and 3 males in this group; one patient had absconded after the first examination. They were all hospital patients. The initial mean hæmoglobin level was 4.6 g.

Diet.—No special diet was given but the hospital on this garden gives a particularly good diet amounting to 2,500 calories and containing a good proportion of protein. Two teaspoonfuls of malt and cod-liver oil were given twice a day for a month.

Hookworm treatment.—In this instance the anthelmintic treatment was given first, one dose of oil of chenopodium and tetrachlorethylene.

Iron.—A dose of $5\frac{1}{2}$ grains of ferrous sulphate was given three times a day for 29 days, a total of $478\frac{1}{2}$ grains.

Response.—Most of the patients showed a slight reticulocyte rise on the tenth day. All showed a marked hæmoglobin increase. On the 29th day the mean hæmoglobin percentage was 11.4 g.

CHART 13.

SYCOTTA.

Per cent reticulocytes
 Per cent Hb. (Hellige)
 Millions R.B.C.

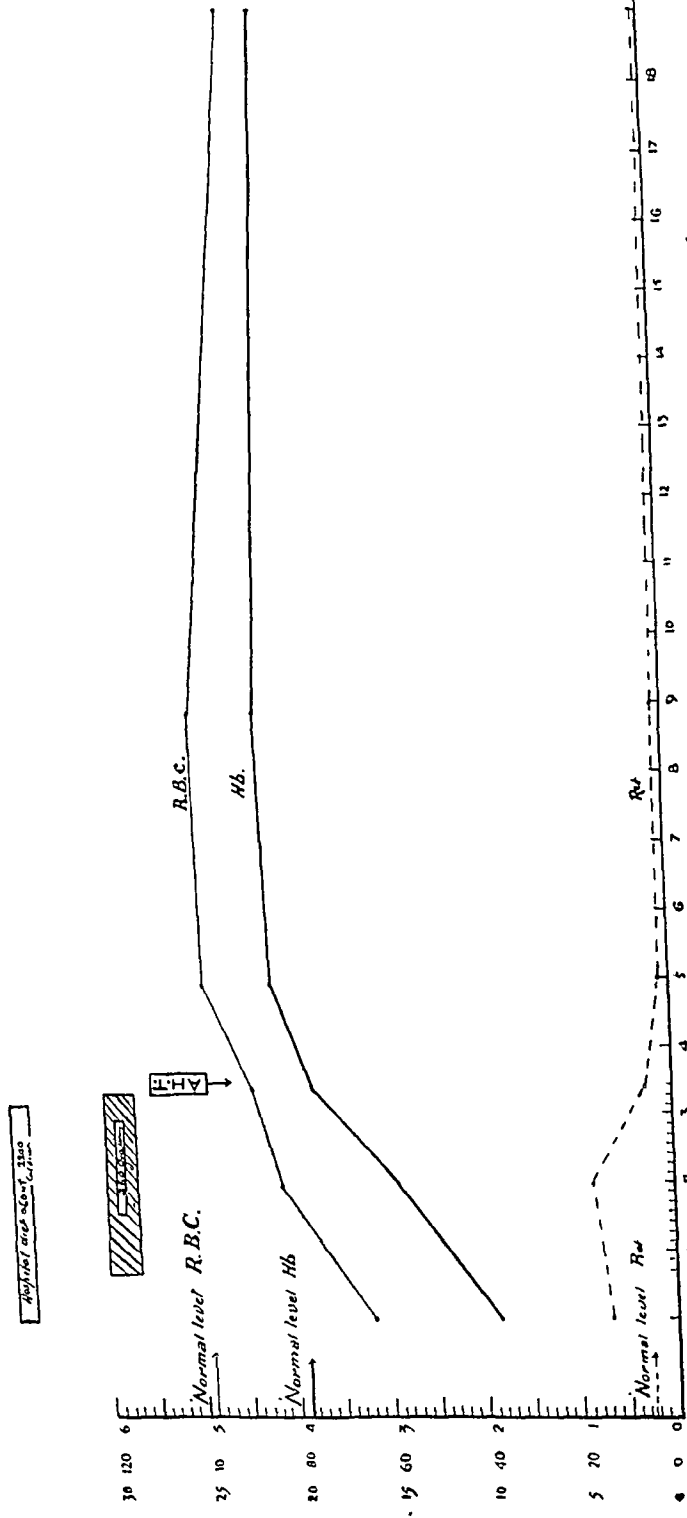
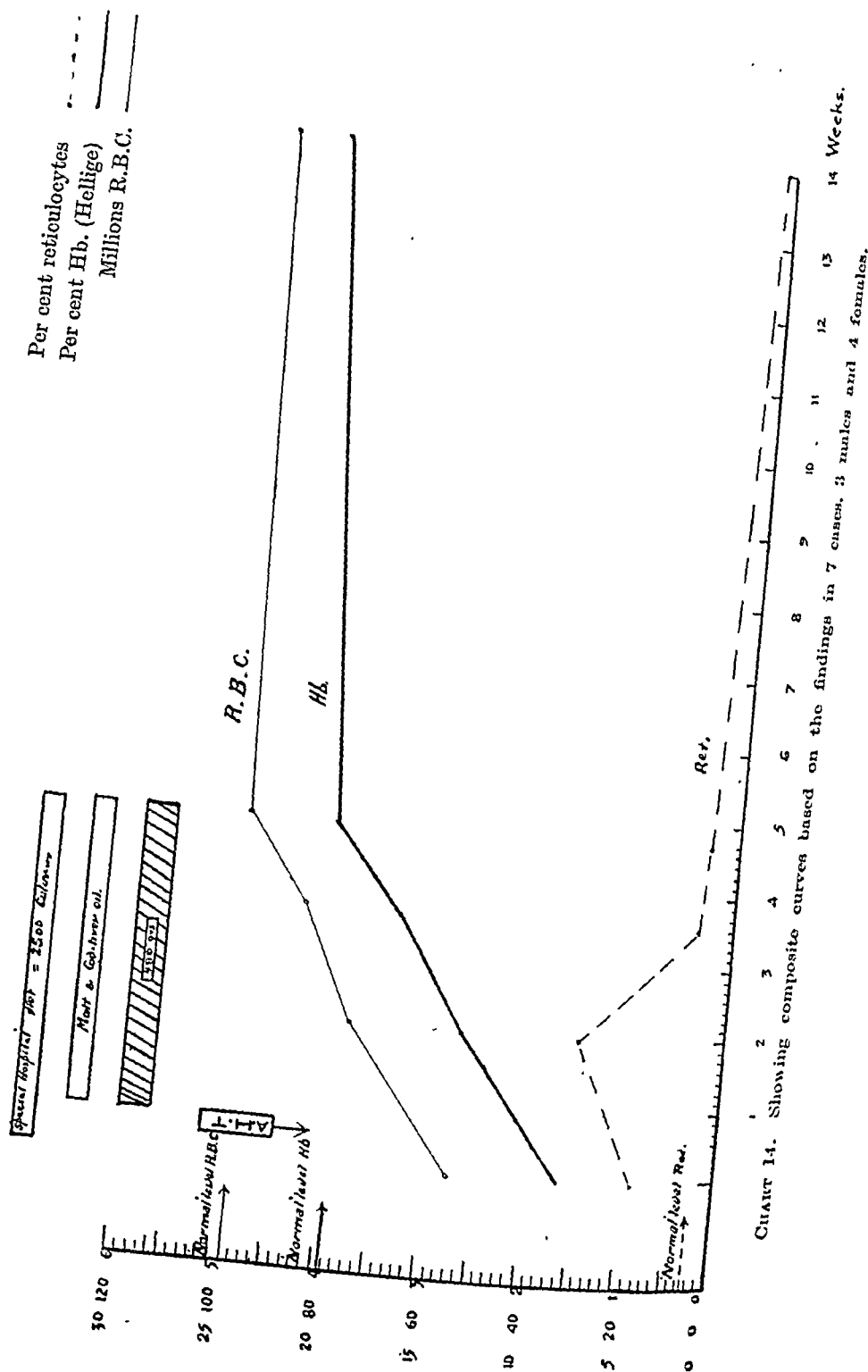


CHART 13. Showing composite curves based on the findings in 6 cases, 2 males and 4 females.

CHART 14.

KATUNIBARI.



Progress.—Sixty-four days later the mean hæmoglobin had risen further to 13.0 g.

Weight.—Only 1 patient had gained weight at the end of a month and 4 had lost weight—mean loss 1.83 lb.

Two had gained weight at the end of observation and the mean gain was nearly a pound.

Kharikateu—Indoor (Chart 15).

There were 4 males and 1 female in this group. They were all hospital patients. The mean hæmoglobin of the group was 4.2 g. before treatment.

Diet.—The patients were on the ordinary hospital diet, of about 2,200 calories. They were given two teaspoonfuls of malt and cod-liver oil daily for three weeks.

Iron.—They received a dose of $5\frac{1}{2}$ grains twice daily for three weeks, amounting to 231 grains.

Response.—At the end of 32 days the mean hæmoglobin was 10.2 g. Most of the cases showed a distinct reticulocyte response on the tenth day.

Hookworm treatment.—A dose of oil of chenopodium and tetrachlorethylene was given immediately the iron treatment was finished.

Progress.—Three months from the conclusion of iron treatment the mean hæmoglobin was 10.0 g.

Weight.—Three lost and 2 gained in the first month but the balance was a mean increase of 1.8 lb. One was not weighed again, 1 lost, 1 remained the same and 2 showed a gain at the last examination—mean total gain 4 lb.

Kharikateu—Outdoor (Chart 16).

There were 3 males and 1 female in this group. They were treated as dispensary cases and continued to work; they received one teaspoonful of malt and cod-liver oil daily but had no food from the hospital.

The initial mean hæmoglobin level was 4.0 g.

Iron.—They were given a single dose of 11 grains of ferrous sulphate before going to work daily for 25 days, a total of 275 grains.

Response.—A reticulocyte response was found in every case on the tenth day. The mean hæmoglobin rose to 9.1 g. on the thirty-second day.

Hookworm treatment.—A single treatment with oil of chenopodium and tetrachlorethylene was given before the iron treatment was commenced.

Progress.—About three months after the conclusion of the iron treatment the mean hæmoglobin level had fallen to 7.7 g.

Weight.—Two had lost and 2 had gained, both at the end of a month and at the final weighing. The mean weights remained unchanged.

KHARIKATEA—Indoor.

Per cent reticulocytes
 Per cent Hb. (Hellige)
 Millions R.B.C.

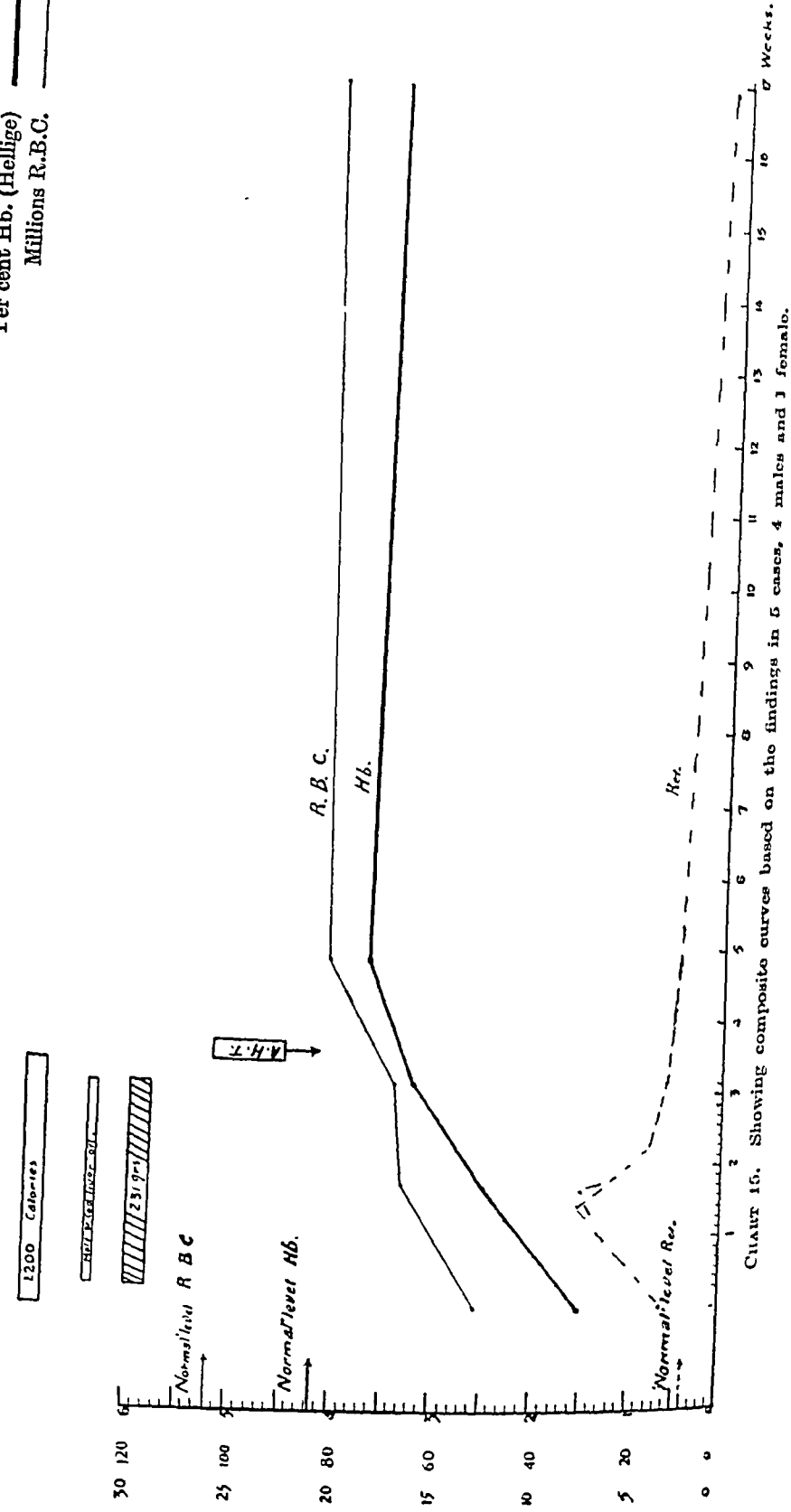


CHART 15. Showing composite curves based on the findings in 5 cases, 4 males and 1 female.

CHART 16.

KHARIKATEA—Outdoor.

Per cent reticulocytes - - - - -
 Per cent Hb. (Hellige) ————
 Millions R.B.C. ————

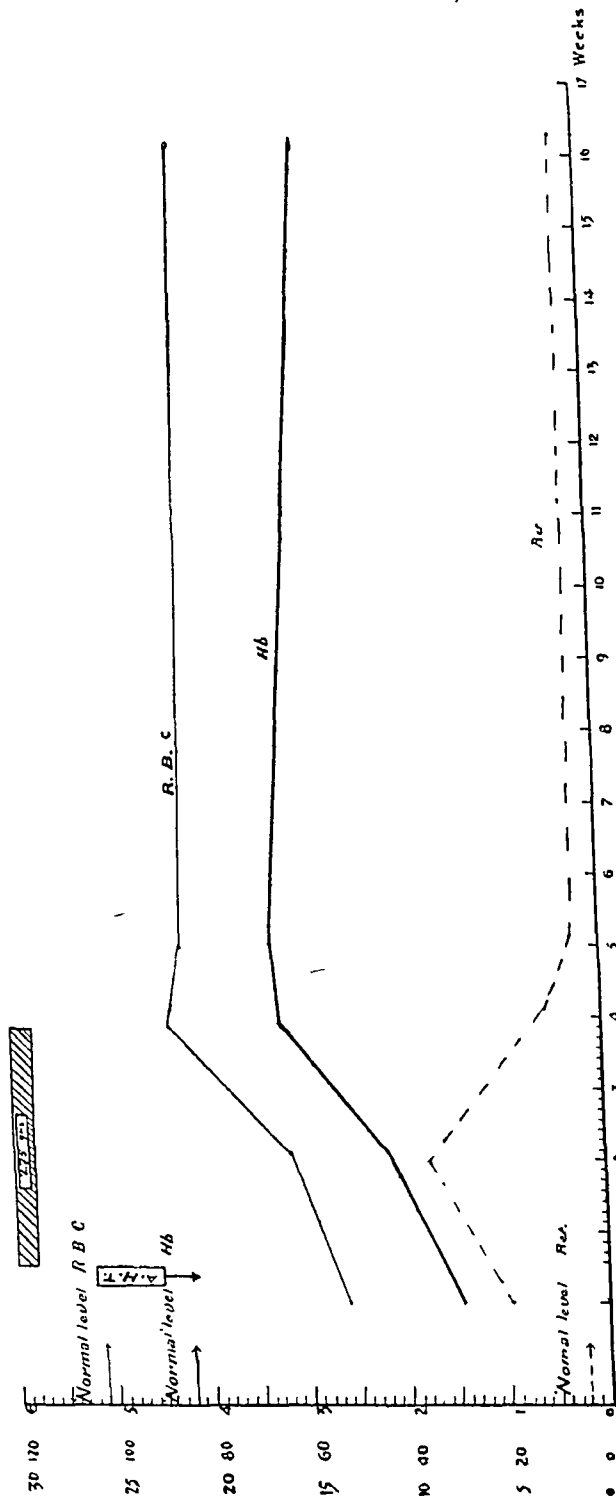


CHART 16. Showing composite curves based on the findings in 4 cases, 3 males and 1 female.

TABLE XVI.

Summarizing results of treatment on different gardens.

Group.	Cases.	Diet in calories.	Iron in grains.	ANTHELMINTIC TREATMENT.		MEAN HÆMOGLOBIN LEVEL AND TOTAL INCREASE BEFORE, AND AFTER DIFFERENT PERIODS FROM COMMENCEMENT OF IRON TREATMENT.			
				Before or after iron.	Number of final egg-counts above 5,000.	Before iron.	A month.	About two months.	About three months.
Nagadholie ..	7	Good, 3,500	522	After	1	4.0	$\frac{10.1}{6.1}$	$\frac{10.9}{6.9}$	$\frac{11.7}{7.7}$
Murmuria ..	8	Good, 3,500	540	After	5	3.9	$\frac{10.3}{6.4}$	$\frac{11.8}{7.9}$	$\frac{11.3}{7.4}$
Sycotta ..	6	Ordinary, 2,200	360	After	0	5.1	$\frac{11.5}{6.4}$	$\frac{11.7}{6.6}$	$\frac{11.0}{5.9}$
Katunibari ..	6	Good, 2,500	478½	Before	1	4.6	$\frac{11.4}{6.8}$	..	$\frac{13.0}{8.4}$
Kharikaten—indoor	5	Ordinary, 2,200	231	After	1	4.2	$\frac{10.2}{6.0}$	..	$\frac{10.0}{5.8}$
Kharikaten—outdoor	4	Poor <2,000	275	Before	3	4.0	$\frac{9.1}{5.1}$	..	$\frac{7.7}{3.7}$

Iron administration in 'normal' coolies.

In order to see if we could raise the level of the hæmoglobin of 'normal' coolies to that of the normal city-dwelling Indians, we gave in a series of coolies, 4 males and 2 females, a daily dose of 11 grains ferrous sulphate for a period of three weeks—total 231 grains.

From the initial mean level of 11.2 g. the hæmoglobin rose to 12.2 g. at the end of 10 days and then receded slightly and was eventually 12.0 g. at the end of two and a half months.

CHART 17.

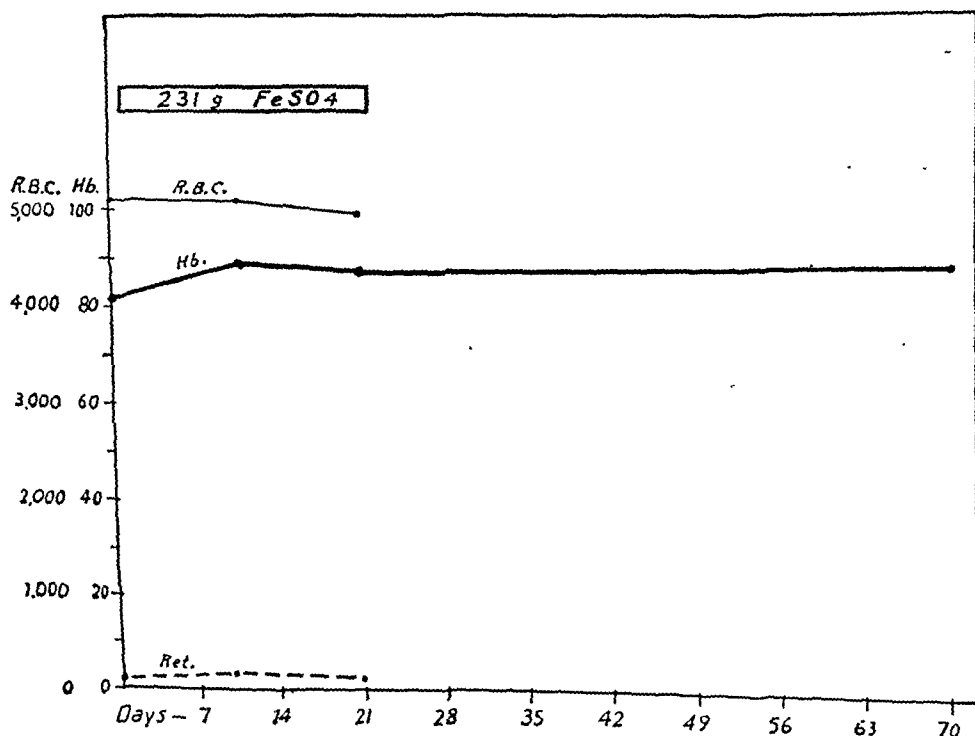


CHART 17. Showing the effect of iron administration to 6 apparently healthy coolies, 4 males and 2 females.

This suggests that there was some slight degree of iron deficiency which was remedied, but that again some limiting factor came into operation before the true normal level was reached.

APPENDIX.

PROGRAMME FOR NAGADHOLIE COOLIES DRAWN UP WITH THE AID OF DR. G. MACDONALD.

Substance.	Weight in chittaks.	GRAMMES OF				Carbohydrates.
		PROTEIN.		FAT.		
		Animal.	Vegetable.	Animal.	Vegetable.	
Eggs, two ..	1.42	9.5	..	8.5	..	1.2
*Milk ..	8.0	15.2	..	16.0	..	21.6
†Chicken, half ..	1.5	21.0	..	3	..	..
Rice ..	10	..	38.5	..	3.4	454.0
Dal ..	1	..	11.4	..	0.22	36.2
Potato ..	1	..	1.0	..	..	10.7
Mustard oil ..	1	..	..	..	56.7	..
Onions ..	1	..	0.74	..	0.06	6.3
Cabbage ..	2.7	..	2.0	..	0.2	13.2
TOTALS ..	..	45.7	53.6	27.5	60.6	543.2
GRAND TOTALS ..	..	99.36		88.1		513.2
Calories ..		407		820		2.225

Add fruit—oranges, bananas, or papaya daily.

A large cabbage can be divided amongst six coolies.

* Milk to be half fresh and half tinned, if the fresh supply is limited.

† The calculation is based on one chicken weighing one pound.

In practice fresh milk was mostly supplied and as mutton was available and cheap, it was frequently given in place of chicken.

DISCUSSION.

The nature of the anæmia.—In our attempt to divide the first series into two separate groups on the hæmatological data we were not supported by statistical evidence, and neither of the only 2 definitely hyperchromic cases showed a high colour index at subsequent examinations. In the second series all but 2 cases were definitely hypochromic, and under treatment these 2 cases came into line with the rest; 32 cases were definitely microcytic. Seven fell within the normal range, and 1 could be classed as macrocytic on the first examination, but of these 8 cases only 1 did not come into line with the rest under treatment.

The patients in both series came from the same population, and, although there were a few cases in the first series with a higher colour index than any in the second series, it seems legitimate to assume that cases in the first series were, as a whole, of the same type as those in the second series about which we have more complete data, especially regarding the response to treatment.

We may, therefore, conclude that with very few possible exceptions the anæmia was of the microcytic-hypochromic type.

In no instance was the anæmia of the pernicious type.

The response to treatment with large doses of iron in 39 out of 41 cases in the second series makes it evident that this microcytic-hypochromic anæmia is an iron-deficiency anæmia.

The cause of this iron deficiency. Actual deficiency in the diet.—It is hard to make any estimate of the iron content of the coolies' diet. It is possibly poor in iron but there is strong evidence to suggest that it is richer in iron than that of many non-anæmic vegetarian communities, as the absence of milk and the low fat consumption are the two most striking features in the dietary. The water in the district contains a large quantity of iron, but the iron is in the ferric state.

Failure of absorption.—The fractional gastric analyses in 87 cases showed the presence of free hydrochloric acid in 80 cases without the administration of histamine. The incidence of achlorhydria is thus lower than in the normal population and the possibility that the iron deficiency is due to failure of absorption on this account is ruled out.

Hookworm infection.—A hookworm infection was demonstrated in nearly every case; in half the cases the hookworm infection was a heavy one. The type of hypochromic microcytic iron-deficiency anæmia from which these patients were suffering is, broadly speaking, the anæmia that is usually associated with hookworm infection.

An examination of random samples of stools taken from the normal coolie population shows almost a hundred per cent infection rate, but only about 14 per cent with a heavy infection (10,000 eggs or more per gramme).

On the other hand, we were unable to show any association between the degree of hookworm infection, as demonstrated by the egg-counts, and the degree of the anæmia.

The actual presence of a heavy infection does not prevent the hæmoglobin from recovering to a point above the normal level of the coolie population, nor

does the persistence of a heavy hookworm infection appear to affect the degree of improvement in individual cases. But in the garden in which the treatment was least effective (Murmuria) the hæmoglobin level tended to fall during the last two months of observation, whereas in another garden (Nagadholie), in which the iron and dietary treatment had been almost the same but the hookworm treatment had been effective, the hæmoglobin continued to rise.

We can summarize our observations by saying that hookworm infection is almost certainly the main factor in the production of anæmia but that, as a normal hæmoglobin level is not incompatible with a heavy hookworm infection, there is probably some other factor that determines why anæmia occurs in some cases and not in others.

Associated factors in the production of anæmia. Malaria.—The spleen index on the gardens from which the coolies came varies from 28 to 39 per cent. The spleen was palpably enlarged in about one-third of the cases, but there is no correlation between the splenic enlargement and the degree of anæmia.

In more than a third of the cases there is hyper-bilirubinæmia; this is not a usual finding in simple hookworm anæmia. It suggests, either (a) that there is some liver dysfunction, of which there is no other evidence, (b) that there is excessive hæmolysis, or (c) that the products of normal hæmolysis are insufficiently re-utilized, because of a deficiency in some other substance essential to normal hæmopoiesis.

Excessive hæmolysis suggests concomitant or recent malarial infection, and it does sometimes occur in cases of chronic splenic enlargement; in the first series there was a comparatively high correlation between hyper-bilirubinæmia and splenic enlargement; this correlation was not however 'significant', nor in the second series was there any evidence of such correlation.

Hyper-bilirubinæmia occurs in pernicious anæmia, where it is due to failure of re-utilization of bilirubin, following hæmopoietic dysfunction associated with deficiency of the hæmopoietic principle, but it does not usually occur in iron deficiency; if, therefore, the suggestion (c) above is to be valid there must be some other deficiency. In the second series, the incidence of hyper-bilirubinæmia is considerably reduced following treatment; this fact lends support to the suggestion that it was due to incomplete re-utilization of the products of normal hæmolysis.

The anæmia associated with acute or chronic malaria does not present the picture of the anæmia with which we are dealing; as in malaria there is only intravascular or intracellular destruction of blood, there is not the loss of iron from the body that occurs in hookworm anæmia.

Diet.—Though none of the coolies are indigent and all earn sufficient money to provide themselves and their families with an ample diet, there is little doubt that the diets on which they live are ill-balanced and deficient in good protein and in fat; there is little evidence of any vitamin deficiency except possibly of vitamin A.

An experiment was designed to see the effect of diet on the blood picture. Eight patients were kept for a period of 28 days on a well-balanced diet with a caloric value of about 3,500. In no one case did the blood picture improve and the mean hæmoglobin in the series was slightly lower at the end of the period.

These patients were then given large doses of ferrous sulphate for three weeks and there was an immediate response; there was a mean increase of 6 g. in the hæmoglobin of the 8 cases within a period of 28 days.

This experiment shows that dietary re-adjustment by itself will not effect an immediate improvement in the blood picture in cases in which anæmia is established. It does not, however, prove that a deficient diet does not produce a state in which the patient is susceptible to a degree of blood loss that in more favourable conditions would be easily compensated.

We must, however, conclude from the evidence here produced that dietary deficiency is not an important factor.

The unknown factor limiting the final hæmoglobin level.—We have shown that, although in nearly all the cases there was a marked response to treatment, there is some limiting factor which prevents improvement beyond a certain level, and that this level is not the level of the better-class, city-dwelling Indian.

Even starting from the higher hæmoglobin level of the 'normal' coolie, improvement beyond a certain point seems to be unattainable.

If we look at the corpuscular values after treatment it will be seen that the MCH and MCV are far below the normal, but that the MCHC approximates very closely to the normal. That is to say, the provision of iron has brought the cell contents up to the usual hæmoglobin concentration, but there is still some factor which interferes with the blood-cell formation.

This deficiency, if it is a deficiency, appears to be common to the whole coolie population as the MCH of the first series of 'normal' coolies (22.2 $\gamma\gamma$) was even less than it is in this series.

The main factor in the production of anæmia in hookworm infection is the loss of blood and all its constituents. Iron is obviously a vital constituent and supplying this will bring the blood up to a certain level, but, in the circumstances of the above experiments, not back to normal.

In the anæmia following malarial infection the loss of blood is from the circulation but not from the body. In the process of hæmolysis the iron is saved and stored; we know something of the subsequent course of the blood pigments, but we know little about the fate of the other blood elements, and we do not know whether certain of these, that are not easily synthesized in the body and whose products are not stored, are not broken down during the process of hæmolysis.

Finally, though we have been unable to show that the anæmia can be cured by the provision of a good diet, we cannot be certain that long periods of deficient diet have not reduced the store of some essential factor for normal hæmopoiesis, so that the body is unable to meet the extra demand which is thrown on it by the long-continued blood loss of hookworm infection, and/or by blood destruction in repeated malarial infections.

THE RESULTS OF DIFFERENT TREATMENT PROCEDURES.

The total dose of iron.—The mean improvement during the first month was fairly constant in the first five groups and bore little relationship to the total iron

dosage, but on the three gardens where the highest dosage was given the improvement was maintained.

The results with the smallest dosage, 11 grains a day for three weeks, were very satisfactory indeed compared with those of treatment by anthelmintic and ordinary iron tonics that was given in the first series.

The effect of diet.—The two groups in which a special diet was given were unfortunately those in which the largest dose of iron was also given, but these results were not quite as good as in the Katunibari group where no special diet was given, though the normal hospital diet is a good one. In the Sycotta group, where a very adequate dosage of iron was given and where the effect of hookworm treatment was very satisfactory, there was an early falling off in the hæmoglobin level, and in the Kharikatea-outdoor group, where the diet was unsatisfactory, the results were poor; on the other hand at Katunibari, where the normal diet of the coolies is good, exceptionally rapid progress was made and maintained. The results in these two groups do suggest, but not very forcibly, that diet has some effect on the maintenance of a high hæmoglobin level.

Hookworm treatment.—In the first series, where for all practical purposes only hookworm treatment was given, except to those treated as hospital patients, the results were far below the worst results in the second series. It is, therefore, apparent that anthelmintic treatment alone will not effect a cure except possibly after a long interval.

It is quite obvious that almost maximal improvement can be achieved without anthelmintic treatment, and that in the presence of a heavy hookworm load a high hæmoglobin level can be maintained for some months (*vide* Murmura). On the other hand, it is quite certain that in time the hookworm infection will cause a recurrence of the anæmia; for example, in the Kharikatea-outdoor group, where a heavy infection was maintained, there was a marked fall in the hæmoglobin level even during the short period of observation of this experiment.

It seems to be a matter of little importance whether the anthelmintic treatment is given before or after the iron treatment from the point of view of the eventual result, but there are certain advantages in giving the anthelmintic treatment to a healthy rather than to an anæmic individual. In this investigation, some of the best results were achieved by giving the anthelmintic treatment first but so also were the worst results [*vide* Katunibari and Kharikatea—outdoor].

SUMMARY.

In 100 cases of anæmia amongst tea-garden coolies there was no case of anæmia of the pernicious type; with very few possible exceptions they were all cases of the microcytic-hypochromic, iron-deficiency type.

The main cause of the anæmia is hookworm infection, but there are almost certainly other associated causal factors; the possibility of these being malaria and dietary deficiency are discussed.

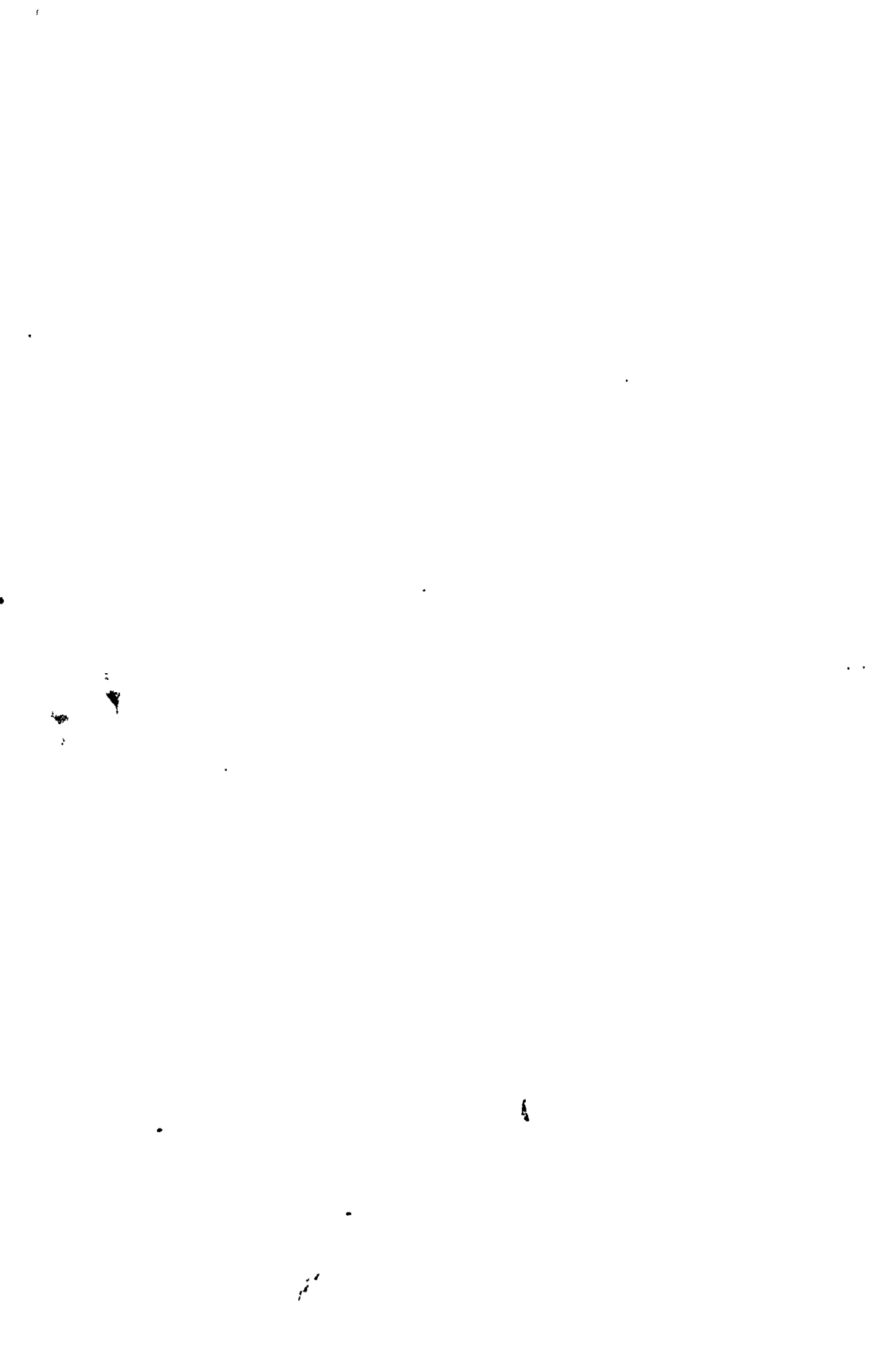
Anthelmintic treatment alone produced little improvement in the anæmia. Dietetic treatment alone also produced no effect on the blood picture.

Large doses of iron (e.g., 18 grains of ferrous sulphate a day for three weeks) caused a rise in the hæmoglobin to the level of the 'normal' coolie population, without any anthelmintic treatment; if effective anthelmintic treatment was then given this level was maintained.

In the iron-treated cases the concentration of the hæmoglobin in the cells reached the normal, but the volume and the hæmoglobin content of the cells still remained about 20 per cent below the normal level, though both values were about equal to those that we have previously shown are the normals for the so-called healthy coolie population.

ACKNOWLEDGMENTS.

Our thanks are due to Dr. D. Manson of the Jorehaut Tea Company for placing a laboratory at our disposal, and to both Dr. George Macdonald and Dr. Manson for their active co-operation, without which this work could not possibly have been carried out, and for many very valuable suggestions during the investigations, to the assistant medical officers in charge of the various tea gardens, and to Mr. L. De who rendered valuable material assistance especially in connection with the counting of hookworm ova. Our thanks are also due to the managers of the gardens on which the coolies were employed, particularly Messrs. Syme and Boswell each of whom took an active interest in the investigation on their respective gardens.



A STUDY OF HUMAN PAROTID SALIVA.

BY

M. A. BASIR, M.B., B.S., PH.D. (Lond.),

AND

T. S. RAMABHADRAN, M.A., L.T.

(*From the Medical College, Vizagapatam.*)

[Received for publication, July 28, 1936.]

IN this paper an attempt has been made to determine quantitatively the relative activity of human parotid saliva with reference to normal mixed saliva.

The parotid saliva for the experiments was from a patient in the wards of the King George Hospital, Vizagapatam, under the care of Captain M. G. Kini. The patient was one Manthi Tata, aged 26 years, Hindu, coolie, married but without children. He had an injury on the right side of the face and neck. Surgical treatment was rendered, but there was a persistent parotid fistula still left. The opening was 2 mm. to 3 mm. in diameter and slightly infected. He had no temperature and was moving about. He was a non-vegetarian, taking ordinary diet, viz., bread, milk, rice, and curry. Whenever he took food, the saliva dribbled from the fistula and it was collected by applying the mouth of a flask over the fistula after rejecting the first flow. About 100 c.c. were collected each time and fresh saliva was used in the experiments.

In this connection we express our grateful thanks to Captain M. G. Kini for kind facilities offered in giving us access to the patient, for the ready and fresh supply of the saliva every day.

I. PHYSICAL PROPERTIES AND SACCHAROGENIC POWER.

(a) *Physical properties.*—

Appearance—slightly opalescent and slimy.

Smell—odourless. Density—1.0064 at 27°C.

Reaction—alkaline—pH 9 to 9.5 using B. D. H. Universal Indicator.

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Appearance—slightly opalescent and slimy.

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Reaction—alkaline—pH 9 to 9.5 using B. D. H. Universal Indicator.

(b) *Method*.—The method of estimating salivary activity adopted was that of Sherman and Walker (1921) with slight modifications. The pure saliva was diluted a known number of times with distilled water according to the needs of the experiment (5 times in the case of mixed saliva and 25 times in the case of parotid saliva). The substrate consisted of 100 c.c. of a 1 per cent dispersion of soluble starch, activated with 5 c.c. of 1 molar sodium chloride and 2.5 c.c. of 0.02 molar disodium hydrogen phosphate, and maintained at a constant pH of 6.64 by the addition of 10 c.c. of phosphate buffer, the temperature being maintained at 38°C. to 40°C. One c.c. of the diluted saliva was allowed to act on this for 30 minutes after which its action was arrested by the addition of 50 c.c. of Fehling's solution. The flask was immersed in a boiling water-bath for 15 minutes. The cuprous oxide formed by reduction was estimated volumetrically by the method of Bertrand (1906). The precipitate, after being filtered through special Gooch asbestos washed with hot water, was dissolved in excess of acid ferrie alum and the reduced iron was titrated against standard potassium permanganate.

Result.—Saccharogenic power—14.5 g. of cuprous oxide per c.c. of pure undiluted parotid saliva.

(c) The relative activities of different specimens of saliva on different forms of starch were also studied by the same method with the following results:—

*Saccharogenic power in grammes of cuprous oxide per c.c.
of undiluted sample.*

Sample.	Starch amylum	Starch soluble.
1. Mixed saliva A .	2.325	2.325
2. Mixed saliva B .	1.700	1.730
3. Parotid saliva ..	14.500	14.500

II. ALLEGED OCCURRENCE OF ACETYLCHOLINE IN HUMAN SALIVA.

Secker (1934) concluded after a series of experiments that the saliva obtained from the sub-maxillary gland of a cat contains a substance resembling very much like acetylcholine in its biological action. Later, Gibbs (1935) repeated Secker's experiments carefully and put forward experimental evidence to show that the substance in the saliva of the cat, which was believed by Secker to resemble acetylcholine, is not acetylcholine itself, but a potent cardiac depressor, the nature of which is still not established.

In the present study with the human parotid saliva, experiments were performed on the frog's heart with a view to determine the nature of the cardiac depressor, if any, alluded to by Gibbs.

Experiments and observations.

A frog's heart was isolated and arranged for perfusion with Ringer's solution, in the usual manner as shown in Fig. 1. Experiments were then performed both with the sample of fresh human parotid saliva and a 1 in 1,000 solution of acetylcholine (acecoline of l'Lezatle et G. Boinet, Paris).

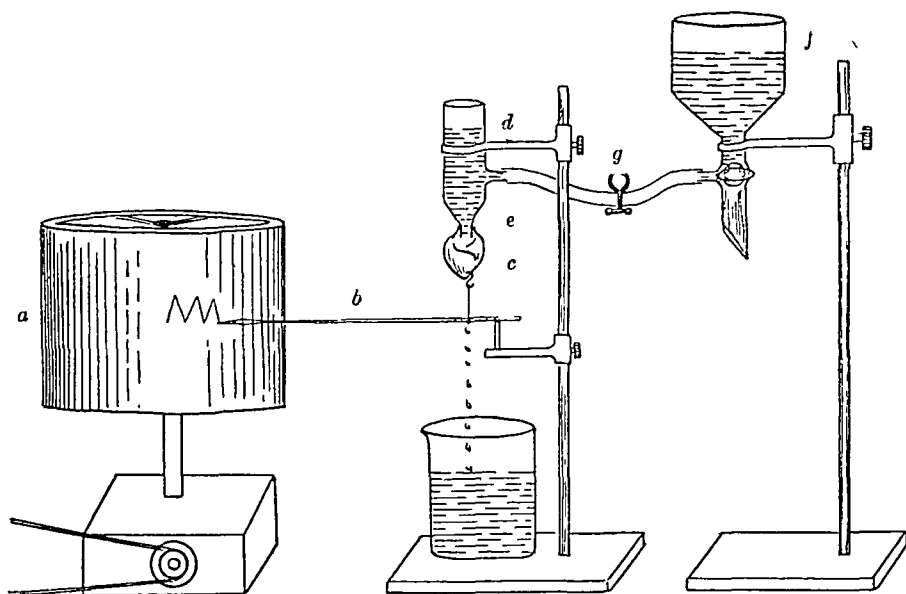


Fig. 1. *Perfusion of isolated frog's heart.*—(a) is the drum on which writes an aluminium lever (b) of Harvard & Co. The lever (b) is attached to an isolated frog's heart (c) by means of a hook. The heart (c) is perfused with frog's Ringer's solution by means of a Syme's perfusion cannula (d) which is tied in the sinus venosus of the heart. The cannula (d) is connected by means of a side tube (e) with a reservoir (f) of the frog's Ringer's solution. The side tube (e) has a pinch-cock (g) to regulate the flow of the Ringer's solution.

(1) *Experiments with the saliva.*—

(a) Five drops of fresh human parotid saliva were added to the Ringer's solution directly into the Syme's perfusion cannula (Fig. 1, d). The amplitude of the heart-beat gradually decreased and finally the heart remained in a slightly contracted condition beating somewhat irregularly. After a short interval, the heart began to beat as before as shown in Fig. 2. So it is evident that the human parotid saliva contains a potent cardiac depressor which inhibits the heart in partial systole.

(b) In another similar experiment, the heart was first subjected to the action of atropine (5 drops of 0·5 per cent solution) and then nicotine (5 drops of 1 per

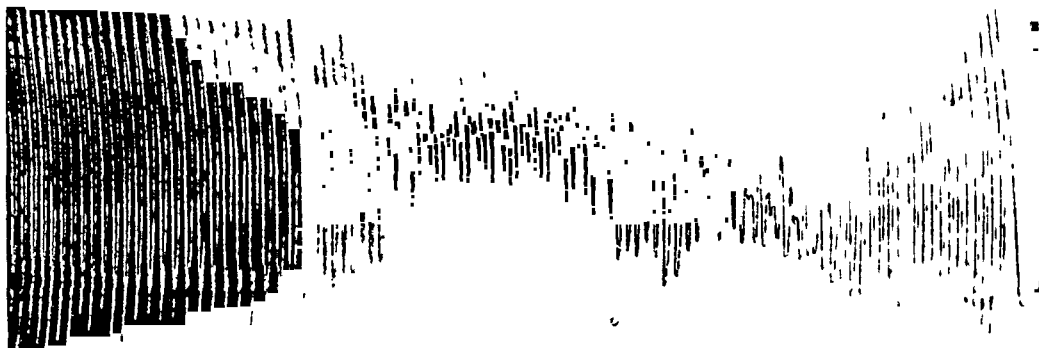


Fig. 2. *Action of human parotid saliva on the frog's heart.*—To be read from right to left. After recording a few normal heart-beats, saliva was added at the mark indicated. The heart was inhibited in partial systole and the beats were small and irregular. Again the heart resumed its normal rhythm.

cent solution) and finally human parotid saliva (5 drops) was added into the Syme's perfusion cannula (Fig. 1, *d*). The results are shown in Fig. 3.



Fig. 3. *Action of the human parotid saliva on the frog's heart, after subjecting the heart first to the action of atropine and nicotine.* To be read from right to left. The heart was inhibited completely in partial systole.

Again the heart was found to be completely inhibited in partial systole. It is at once evident that atropine and nicotine have not modified the action of the cardiac depressor present in the saliva, but on the other hand have accentuated its action.

(2) *Experiments with the solution of acetylcholine (1 in 1,000).—*

(a) To another preparation of frog's heart, as in the above perfusion experiment, 5 drops of acetylcholine (1 in 1,000) were added to the Syme's perfusion cannula (Fig. 1, *d*). The heart was inhibited in diastole as shown in Fig. 4. Then gradually the heart began to beat with the usual rhythm.



Fig. 4. *Action of acetylcholine (1 in 1,000) on the frog's heart.*—To be read from left to right. At Ac, the acetylcholine solution was added. The heart was inhibited in diastole. Gradually the heart regained its usual rhythm.

(b) The same experiment as above was repeated with another frog's heart, but it was subjected at first to the action of atropine (5 drops of 0.5 per cent solution) and then nicotine (5 drops of 1 per cent solution) and then finally acetylcholine (5 drops of 1 in 1,000 solution) was added to the Syme's perfusion cannula (Fig. 1, *d*). As shown in Fig. 5, there was no change in the heart-beat brought about by acetylcholine.

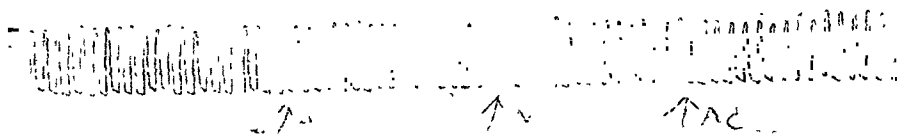


Fig. 5. *Action of acetylcholine (1 in 1,000) on the frog's heart.*—After subjecting the heart at first to the action of atropine and nicotine. To be read from left to right. At A, atropine solution, at N, nicotine solution and at Ac, acetylcholine solution were added in succession. No effect was noticed in the heart-beats.

Evidently then, the preliminary treatment of the heart with atropine and nicotine had annulled the inhibitory action of the acetylcholine. On the other hand, in one of the experiments, there was an actual increase in the rate and amplitude of the heart, showing thereby that the action of acetylcholine can be reversed by such preliminary treatment of the heart with atropine and nicotine.

DISCUSSION.

The human parotid saliva, therefore, contains a powerful cardiac depressor whose action is not modified by atropine or nicotine (Fig. 3). This depressor

(b) In another similar experiment, the heart was first subjected to the action of atropine (5 drops of 0.5 per cent solution) and then nicotine (5 drops of 1 per

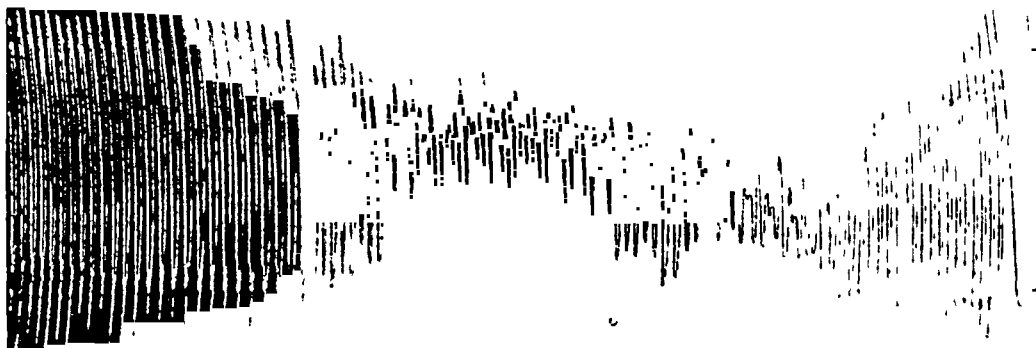


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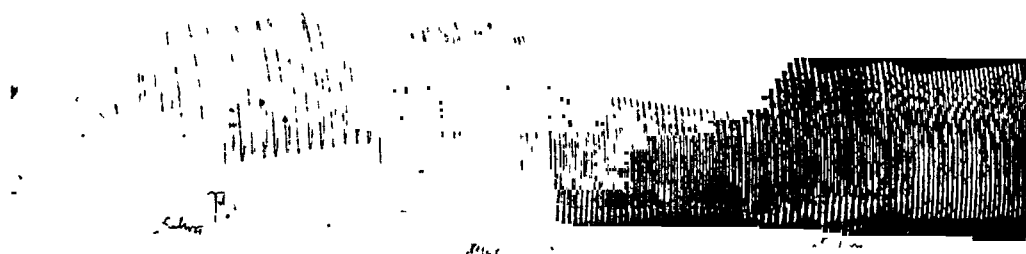


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Again the heart was found to be completely inhibited in partial systole. It is at once evident that atropine and nicotine have not modified the action of the cardiac depressor present in the saliva, but on the other hand have accentuated its action.

THE POSSIBLE INFLUENCE OF SOLAR RADIATION ON THE PRODUCTION OF CATARACT IN CERTAIN DISTRICTS OF SOUTHERN INDIA: A PRELIMINARY INVESTIGATION.

BY

LIEUT.-COLONEL R. E. WRIGHT, C.I.E., I.M.S.,

Professor of Ophthalmology, Government Ophthalmic Hospital, Madras.

(Inquiry under the Indian Research Fund Association.)

[Received for publication, July 20, 1936.]

It has been held for a long time that cataract is much more common in India than in Western countries. The idea that exposure to excessive solar radiation was largely responsible was a natural corollary. Of recent years scientific interest in these views has been stimulated by Vogt's (1931) work on the experimental production of cataract by infra-red (ultra-red) rays. He showed that the near infra-red region (2,400 to 750 millimikra) was very active in this respect, whereas the more distant infra-red and ultra-violet radiations were not damaging to the lens. The well-known cataracts met with in glass-blowers and iron-puddlers are also a product of the absorption of infra-red radiation. These have certain peculiar features some of which are readily recognized. Mulock-Houwer (1934) recorded that lenticular changes which he and Mingelen had observed in Europeans in Java showed a great similarity to glass-blowers' cataract. He found that in the Javanese, cataract of a caloric type is not so common as in Europeans living in Java, and inferred that the lenticular change in the latter is an indication that exposure to great intensities of solar radiation is a definite ætiological factor in persons not protected by heavy iris pigmentation. He suggests that in British India the ultra-red radiation may be more active, and constitutes an important ætiological factor in cataract production even in Indians; especially in areas which, unlike Java, are dry and in which atmospheric moisture does not partially absorb the infra-red radiation reaching the earth's surface.

Long experience of cataractous conditions did not lead me to think that the characteristic features of infra-red absorption were sufficiently common to warrant the view that the part played by thermal energy was important enough in South India to account for an exceptionally high frequency in the occurrence of cataract.

In order to get further information on these matters this investigation here recorded was undertaken.

At the outset one had to assume it probable that solar radiation, (and its infra-red extension with which we are now concerned), reached the earth in greater intensities in hot, dry, cloudless districts, and in lesser intensities in moist and more cloudy areas. This presumption may not be wholly justified since the various atmospheric factors influencing the solar radiation is a problem full of difficulties. It is commonly accepted, however, that aqueous vapour in the atmosphere absorbs a considerable amount of the infra-red radiation and prevents it reaching the earth.

Two districts were chosen, Bellary (Deccan) and Calicut (Malabar), in one of which the outdoor labourer was liable to be exposed to greater intensities of the infra-red solar radiation than in the other. The choice of districts was limited inasmuch as it was necessary to obtain the help of trained observers. In each district some 2,000 outdoor labourers between the ages of 40 and 60 were inspected. They were taken at random and examined for evidences of cataractous change. In the early or incipient cases, posterior sub-capsular change was looked for. The presence of this feature does not furnish certain evidence of a thermal causation, but its absence practically negatives this ætiology.

It was impossible under the conditions of the investigation to have corneal microscope observations made, to determine if the changes in the lens capsule (separation of the zonular lamella), said to be peculiar to caloric cataract, were present; nor yet could it be noted with certainty if the posterior sub-capsular change characteristic of the absorption of infra-red rays was the type actually observed. There are other posterior sub-capsular or saucer-like cataractous changes not due to infra-red absorption with which the former is liable to be confused unless subjected to examination with a corneal microscope. It is possible, however, by ordinary clinical methods to note the presence or absence of posterior sub-capsular changes in the majority of incipient cases and thus determine a group which would include incipient cataracts of infra-red origin. A relative frequency or infrequency of posterior sub-capsular cataract, including all types, might reasonably be held to discriminate for or against the ætiological importance of infra-red radiation.

A statement of the collected figures is given in the Table opposite.

In attempting to draw conclusions from the figures here presented there are a number of points which must be taken into consideration as detracting from their value. Amongst others the following are important:—

- (a) The ages 40 to 60 are presumptive. Few peasants know their age. It may be inferred that the average individual in these groups looked older than his or her actual age. The term senility is not a convenience when considering cataract in India, since it is associated in the minds of many with great age rather than with unknown noxious influences of various sorts in greater or lesser amounts acting over a variable time period. We are apt to speak of premature senility. Such a condition would probably be common amongst the individuals of our groups.

TABLE.
The occurrence of cataract in outdoor workers between the ages of 40 and 60 in
South Indian districts.

Place.	Number of persons examined.	IMMATURE OR INCIPIENT CATARACT.		Mature cataract.	Total cataract.	Incidence of cataract.	Incidence of posterior sub-capsular cataract.	REMARKS.
		Posterior sub-capsular.	Other than posterior sub-capsular.					
I. DECCAN (Bellary).—								
Average maximum temperature, 93.3°F.	2,000	19	276	94	389	1 in 5.1 approx.	1 in 15.5	The figures do not reveal anything likely to be of importance in the rate of occurrence amongst males and females respectively in the Deccan group.
Average mean temperature, 82.0°F.								
Relative humidity, 61 per cent.								
Rainfall, 1.6 inches								
Number of rainy days, 2.6								
II. MALABAR (Calicut).—								
Average maximum temperature, 86.7°F.	2,000	5	461	123	589	1 in 3.4	1 in 93.2	In the Malabar group a note of sex was only made in the case of 743. The figures do not suggest any great divergence in the rate of occurrence as between males and females, but the total number of females examined is too small for comparative purposes.
Average mean temperature, 80.7°F.								
Relative humidity, 83 per cent.								
Rainfall, 9.75 inches								
Number of rainy days, 8.6								

The incidence of cataract is very high. It is higher in Malabar where the factor of infra-red radiation is less operative.

The posterior sub-capsular types are more common in the Deccan figures where infra-red radiation may be expected to play a more active rôle.

- (b) In Group II (Malabar) four observers are responsible for the figures, whereas in the other group one observer saw all the cases. Errors of observation are more likely to have been recorded in Group II, but it is improbable that the difference in incidence of posterior sub-capsular cataract can be accounted for in this way to any great extent.
- (c) Of the posterior sub-capsular cataracts in either group, one cannot say with certainty if any are directly due to infra-red radiation.

In spite of various sources of error, the data appear to show :—

1. That the incidence of cataract between the ages stated is unexpectedly high in each group (1 in 5.1 and 1 in 3.4, respectively).
2. It is higher in the area where one might have expected the effects of infra-red radiation to be less active.
3. The occurrence of posterior sub-capsular cataract is very much less common in the area where presumably there is a lesser exposure to that portion of the infra-red radiation known to be absorbed by the lens.
4. Although one cannot conclude that infra-red radiation is a deciding factor in determining the greater frequency of posterior sub-cortical cataract in the area in which it might *prima facie* be expected to be more active, it is significant that this greater frequency appears (1 in 15.5 as compared with 1 in 92.2).
5. The relative infrequency of posterior sub-cortical cataract in the Malabar area appears to show that infra-red radiation is ætiologically unimportant as compared with other causative agents.

This investigation, taken in conjunction with the fact that bio-microscopical observations on posterior sub-capsular cataracts in Madras during the past ten years have revealed few in which characteristic infra-red changes were present, would seem to indicate that in South Indians solar radiation is probably not an important factor in determining the high frequency of cataract which evidently exists.

ACKNOWLEDGMENTS.

My acknowledgments are due to the following for collecting the data on which this paper is based :—

Dr. P. Narayana Menon, retired Civil Assistant Surgeon, Mr. D. Narasimha Raju, Sub-Assistant Surgeon, Mr. M. Anandan, Sub-Assistant Surgeon, Mr. P. V. Karunakaran Nair, Honorary Sub-Assistant Surgeon, Dr. P. Balakrishna Menon, and to Lieut.-Colonel Leslie, I.M.S., for his co-operation.

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ON THE MALE TERMINALIA OF *CHRYSOMYIA* *MEGACEPHALA* AND *C. BEZZIANA*.

BY

D. N. ROY, M.D., D.T.M. (Cal.).

(From the Department of Medical Entomology, School of Tropical Medicine,
Calcutta.)

[Received for publication, July 28, 1936.]

PATTON and CUSHING (1934) have given a comparative description of the terminalia in both the males and females of some of the species of *Chrysomyia*, and these studies have enabled them to compose a reliable practical guide for the classification of the genera. I now wish to review the Oriental *Chrysomyias* in this respect but in this paper observations will be confined to two species *C. bezziana* and *C. megacephala* only, and I intend to state only the salient characters whereby the differentiation between the males of the two species can be effected.

In conducting these observations some difficulty was experienced at first over the features of some flies which resembled *C. megacephala* in all their external characters, except that their colour was a metallic green as against the dull bluish-green of *C. megacephala*, and in which there was also a difference in size and in their natural breeding habits. Earlier too in the investigation it was thought that there was a well-marked difference in their terminalia and it was believed that they represented a new species. But after a large number of these flies had been examined it was found that they were *C. megacephala* and that this species exhibited a great deal of variation in the disposition of bristles on the paramere.

For the preparation of the flies they were treated with caustic potash, cleared in carbolic acid, and then dissected in clove oil. In the descriptions of the different parts Patton's (1932) terminology has been used. All the preparations of the male terminalia have been made in the same position and the drawings executed with the camera lucida (see Plates XXXI and XXXII).

My conclusion is that no appreciable difference can be detected in the character of the 9th coxite and anal cercus or in the shape of the 9th tergo-sternum, and, in the main structural details, the phallosome of *C. bezziana* and of *C. megacephala* resemble each other.

The parameres alone present distinctive characters in the two species. The number of long hairs present on the ventral edge of the anterior paramere varies: in *C. megacephala*, the usual number is 5 but 3, 4, and 6 have been noticed, while in *C. bezziana* the number is constantly 4, so that this feature itself is not invariably diagnostic.

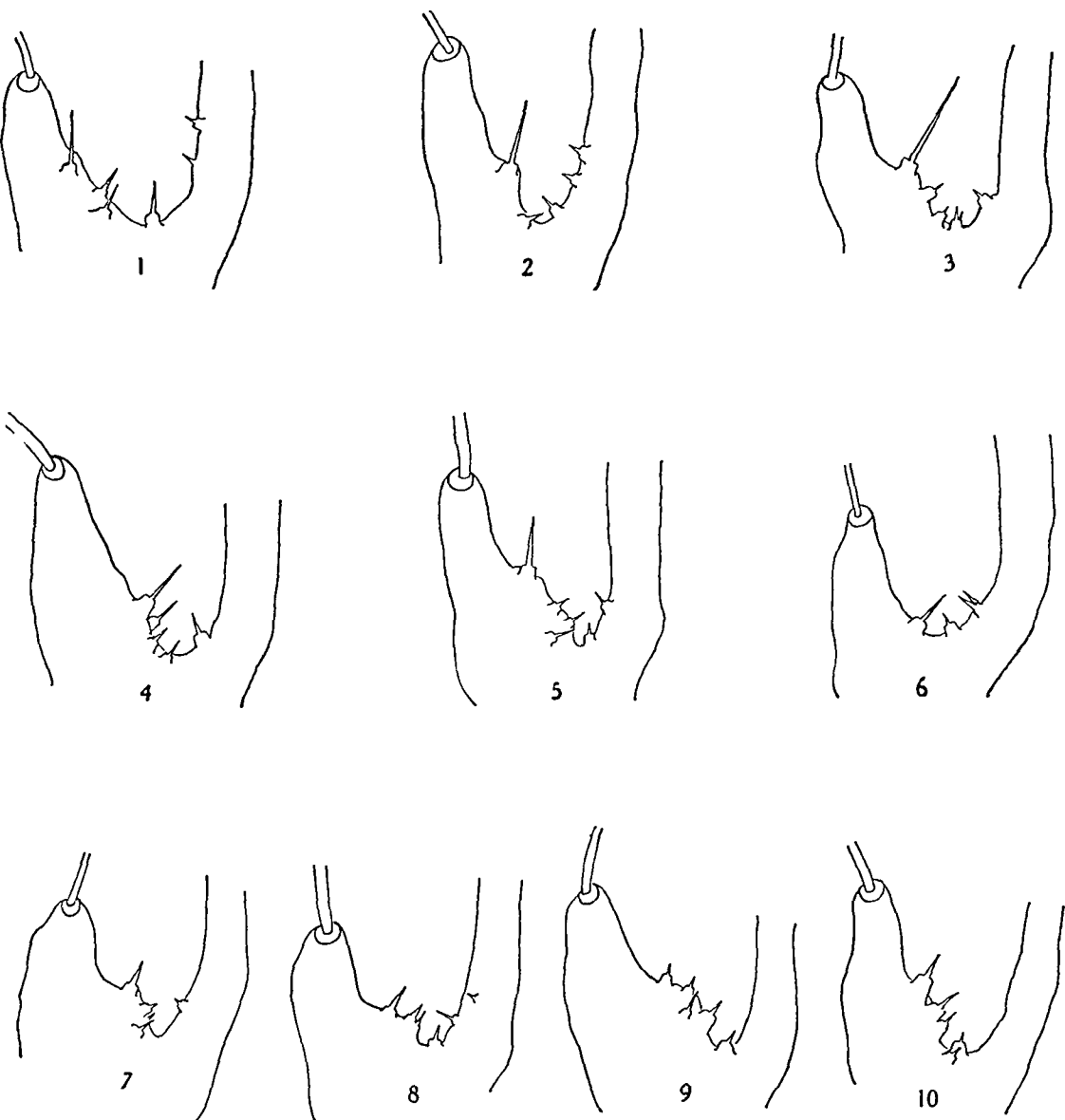
The position of the attachment of the sensory spines on the posterior part of the paramere varies widely in *C. megacephala*, as will be evident from the illustrations. The first spine on the accessory process is usually placed at the junction of the outer third and inner two-thirds, it projects out considerably from the body and is placed on a well-marked pedestal. In *C. bezziana*, on the other hand, it is placed much lower down, at the concavity at the junction of the accessory process and the shaft of the posterior part of the paramere. All the spines are considerably better developed in *C. megacephala* than in *C. bezziana*.

It will thus be seen that an intensive study of the male terminalia of higher diptera, along the same lines as Patton, is likely to reveal characters of 'specific' distinction, a matter which was first pointed out by him.

REFERENCES.

- PATTON, W. S. (1932) *Ann. Trop. Med. Parasit.*, **26**, No. 3, pp. 347-405.
PATTON, W. S., and CUSHING, E. C. (1934) *Ibid.*, **28**, No. 1, pp. 123-130.

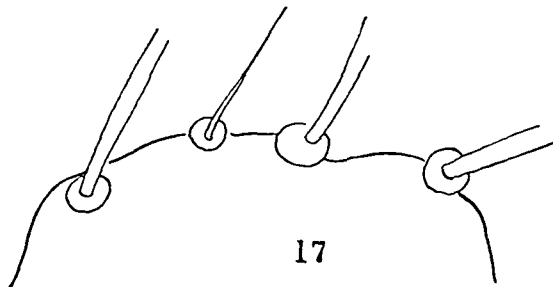
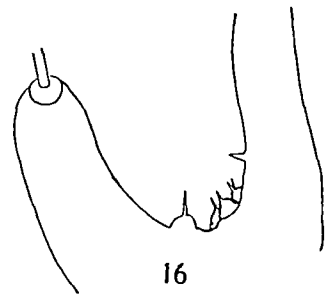
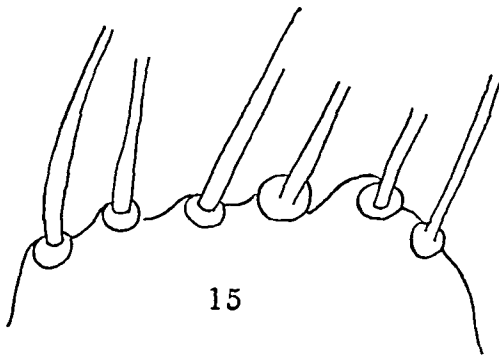
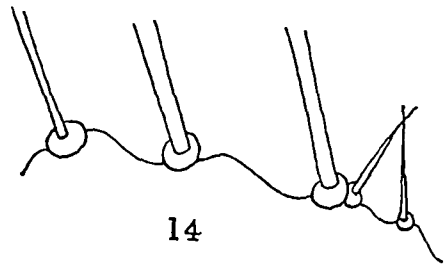
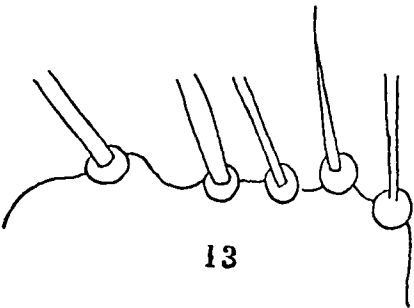
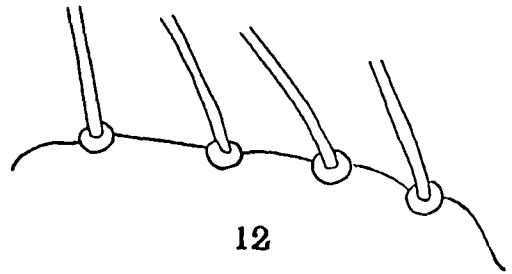
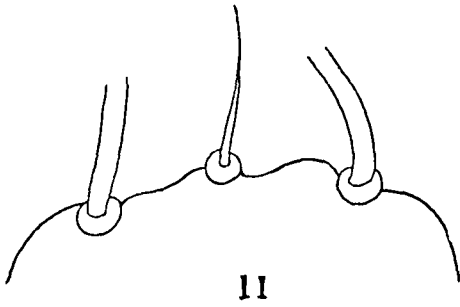
PLATE XXXI.



Figs. 1 to 10.— Posterior part of the paramere of one side, showing the variation in the position and length of the spines in *C. megacephala*.

del. Roy.

PLATE XXXII.



Figs. 11 to 15.—Showing the number of hairs on the ventral edge of anterior paramere in *C. megacephala*.

Fig. 16.—Showing the posterior part of the paramere in *C. bezziana*.

„ 17.—Showing the number of hairs on the ventral edge of anterior paramere in *C. bezziana*.
del. Roy.

NOTES ON ANTIRABIC IMMUNIZATION: A CORRECTION.

BY

LIEUT.-COLONEL G. COVELL, M.D., D.P.H., D.T.M. & H., I.M.S.,
Late Officiating Director, Pasteur Institute of India, Kasauli.

[Received for publication, December 12, 1936.]

A PAPER entitled 'Notes on Antirabic Immunization' by Covell, McGuire, Stephens and Lahiri appeared in the *Ind. Jour. Med. Res.*, **24**, 2, pp. 373-388. In it was given an account of certain experiments which were carried out or completed during the two years in which the present writer was officiating as Director of the Pasteur Institute of India.

At the beginning of Section 1, which dealt with the use of antirabic serum and live fixed virus as adjuncts to treatment with carbolized vaccine, the following sentence occurred:—

'The results of previous experiments in this connection were reported by Shortt *et al.* (1935). Those now recorded represent a continuation of the same investigations'.

The reference was to a paper by Shortt, McGuire, Brooks and Stephens published in the same *Journal*, **22**, 3, pp. 537-556.

A similar sentence should have been inserted at the head of Sections 5, 6, and 7, which gave an account of experiments in connection with the keeping properties of carbolized vaccine, the relationship between complement-fixation tests and immunity to rabies, and the immunizing value of a rabies virus fixed in the dog.

These experiments were all initiated by Lieut.-Colonel Shortt, I.M.S., to whom I wish to make my acknowledgments.

NOTICE.

The following has been received for announcement :—

—Editor.

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THE object of this Bureau is collection on as wide a scale as possible of material dealing with human Genetics. Later, the tasks of analysis of material and distribution of the information available will be added.

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The Council would be grateful to receive all available material from Institutions and individuals, furnishing well-authenticated data on the transmission of human traits whatever these may be. Pedigrees are particularly desired; twin studies and statistical researches are also relevant. As research workers and others who send in material may in some cases wish to retain the sole right of publication (or copyright) those who so desire are asked to accompany their material with a statement to that effect.

Material should be given with all available details in regard to source, diagnostic symptoms, and the name and address of the person or persons who vouch for accuracy. All such details will be regarded as strictly confidential.

Reprints of published work would be most acceptable. Further, many authors when publishing material may also have collected a number of pedigrees which they have been unable to reproduce in detail. It is the object of the Council that such records, by being included in the Clearing House, should not be lost.

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Announcements in regard to the services undertaken by the Bureau will be published from time to time.

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J. A. Fraser Roberts, L. E. Halsey (Hon. Treasurer),
C. B. S. Hodson (Hon. Gen. Secretary).

NOTICE.

Fourth Course of Post-graduate Instruction in Malariology, under the auspices of the League of Nations, Singapore.

April, 1937.

THE League of Nations is arranging for a fourth Course of Instruction in Malariology which will commence at the King Edward VII College of Medicine at Singapore, on Monday, the 19th April, 1937.

The object of the Course is to complete the training of medical practitioners who are engaged, or intend to be engaged, in the work of the malaria control in their own countries. The Course will thus be of interest not only to Governments and Municipal authorities, but also to all medical men practising in Eastern countries, particularly those engaged in estate work.

The Course will comprise two stages, one consisting of theoretical, clinical, and laboratory studies with practical demonstrations, and the other devoted entirely to practical field studies.

The first stage will commence on 19th April and continue until the end of May. It will be carried out partly at the King Edward VII College of Medicine and partly at the Tan Tock Seng Hospital, Singapore.

The second stage will commence at the beginning of June, when candidates will proceed in groups either to Indo-China, the Netherlands Indies, or Malaya, where they will have the opportunity to study the routine work of malariologists and the application of control measures under field conditions. This stage of the Course will last 14 to 21 days.

Conditions of admission.—The Course is open to medical practitioners, from whom applications for admission will be received up to the 27th February, 1937.

Fee.—The fee for the whole or any part of it is Seventy-five Straits Dollars (\$75), payable in advance.

Fellowships.—The League of Nations is making available a limited number of Fellowships which will only be granted to candidates nominated by their Governments or semi-official institutions.

General.—Further information concerning the conditions governing the granting of fellowships or admission to the Course will be supplied on application to the Director, League of Nations Eastern Bureau, 336 River Valley Road, Singapore.

—Editor.

SYLLABUS.

THEORETICAL, CLINICAL AND LABORATORY STUDIES, WITH
PRACTICAL DEMONSTRATIONS.**1. Entomology :—**

This section of the Course will comprise lectures and practical laboratory work spread over a period of four weeks and lasting for approximately 58 hours.

Syllabus.—The detailed morphology of anopheline imagines, pupæ, larvæ, and ova. The œcology of imagines, larvæ, and ova; physical and biotic autœcology, synœcology. The genus *Anopheles*, its classification and affinities; the species and categories below the species; geographical and seasonal distribution; the characteristics of the principal carriers of the world. Susceptibility; influences determining the efficiency of carriers. The determination of species in all stages; the use of keys and original descriptions in a study of the Eastern fauna. The technique of laboratory and field investigations.

2. Haematology and Protozoology :—

A series of lectures and laboratory classes comprising ten lectures and seventeen hours' practical laboratory work.

Syllabus.—Blood cytology, staining methods, enumeration methods. General consideration of the protozoa with particular reference to the sporozoa (plasmodium, hæmoproteus, etc.). Detailed study of the malarial parasites. Malaria in birds, monkeys, and other animals. (A more detailed syllabus will be available before the Course begins.)

3. Pathology :—

Immunity under experimental conditions. A series of lectures, demonstrations, and laboratory studies lasting ten and a half hours.

Syllabus.—Nature of the malarial paroxysm. Pathogenicity of malaria parasites—virulence of parasite and susceptibility of host. Changes in organs and tissues in acute pernicious, in benign, in chronic and latent malarial infections, and in super-infections—correlation with clinical syndromes. Toxins, changes in the blood; cellular, biochemical and immunological. Immunity—natural immunity and acquired immunity (resistance, tolerance, premunition). Cellular basis for immunity. Antibodies. Factors influencing immunity—therapy, splenectomy, blockade, etc. Duration of immunity. Relapse. Spleen size and parasite prevalence in relation to immunity. Infection and super-infection under experimental conditions. Strains of malaria parasites—specificity; importance in relation to specific therapy and epidemiology.

4. Clinical :—

A series of lectures and clinical demonstrations extending over a period of four days.

Syllabus.—Demonstration of selected cases and discussion. Relapses and resistance to malaria; provocation of attacks. Induced malaria; delayed manifestations; complications and sequelæ. Toxicology in relation to the use of quinine and other drugs. Determination of what constitutes a cure. Blackwater fever.

5. Therapeutics :—

A series of six lectures of one hour each.

Syllabus.—Plasmoquine, Fournau 710, Atebrin, arsenicals, and other preparations. Pharmacology and toxicology; dosage and administration. Therapeutic effects; effects on the various stages of the parasites; effects on relapses. The present status of synthetic drugs in comparison with quinine and its derivatives.

6. Epidemiology :—

A series of 12 lectures of one hour each.

Syllabus.—Methods and interpretation of results; measurement of malaria (spleen rate, parasite rate, sporozoite rate, etc.) and the estimation of its prevalence and intensity; statistical analysis of

data. Spleen—theory. Endemicity, epidemicity, and pandemicity, their characteristics and the factors involved. The prediction of epidemics. Topographical, climatic, and social factors. Anopheles *sine malaria*.

7. Control :—

A series of lectures and lecture-demonstrations will be given occupying a minimum period of 60 hours. Field demonstrations on urban malaria control will be arranged through the courtesy of the Municipal Health Officer, Singapore. Opportunity will be given to students to familiarize themselves with field conditions in association with the theoretical lecture-courses in Entomology and Epidemiology.

Syllabus.—The history of malaria control in Singapore. The organization of anti-malaria services in urban and rural areas. The classification and application of malaria-control measures. The importance of drainage. Species control and the choice of methods suitable to a particular locality. The influence of biological knowledge on the control of malaria. The limitations and uses of larvicides. Malaria surveys; the measurement of malaria in the field. The relative cost of malarial endemicity. Temporary (recurrent) and permanent control measures, their efficiency, cost, and financial benefit. Anti-malaria legislation and propaganda.

8. Special Lectures :—

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A STUDY OF THE VIBRIO GROUP AND ITS RELATION TO CHOLERA.

BY

COLONEL J. TAYLOR, M.D., D.P.H., I.M.S.,

S. R. PANDIT, D.B., M.B., B.S.SC.,

Ind. Jour. Med. Res., **24**, 4, April, 1937.

CORRIGENDUM.

In paper entitled 'Adsorption of Antigens by Antibodies or vice versa. Part III' by B. N. Ghosh and N. N. Ray, *Ind. Jour. Med. Res.*, **24**, 3, January, 1937, p. 629, Table IV, for 'tetanus' read 'diphtheria'.

Mackie and Storer (1918) working in Egypt isolated from cases of choleraic diarrhoea vibrios inagglutinable with cholera high-titre sera, to which they gave the name 'paracholera' vibrios and concluded that 'it is probable that this group comprises a number of different types and species' and that the 'evidence is strongly in favour of these strains being causally related to the cholera-like disease with which they were associated'.

Similarly, Greig (1916) investigated a series from Calcutta water sources and from 31 vibrios defined six serological groups.

The serological examinations made by these and other workers were however not specifically directed to an examination of the heat stable ('O') antigen, although the method of examination probably resulted in readings which would indicate it (Gardner and Venkatraman, 1935). The value of the separate estimation of 'O' agglutination in the course of the examination of vibrios was shown in the reports of certain workers, notably Bruce White, in the course of the trials which had been carried out with sera prepared in connection with an investigation directed towards the production of a standard cholera agglutinating serum, and which are embodied

in the collected series of reports published by the Office International d'Hygiene Publique (1934).

The first detailed study of vibrios of a variety of sources of origin, including strains other than typical *V. cholerae*, was carried out by Gardner and Venkatraman (*loc. cit.*) who, on the results of examination of a series of 47 strains other than the classical type, defined five 'O' serological groups in addition to the typical *V. cholerae*, 20 strains being classified in the five groups and 27 being serologically individual.

The present paper is based on an investigation into the characters of vibrios carried out as part of a larger scheme with which other cholera inquiries which have been established in India were associated. At Kasauli a detailed study of the serological and biochemical characters of recently isolated strains was undertaken. The object of this portion of the Inquiry was the examination of strains isolated from cases of clinical cholera, healthy carriers and water from endemic and non-endemic areas in India, for the purpose of ascertaining whether vibrios of other than the classical type and of any consistent nature could be ætiologically associated with the disease, the distribution of such strains in different areas and in different sources and their possible relationship to the classical type. It was intended that this should form a basis for future bacteriological and epidemiological studies in India, both in endemic and non-endemic areas. No attempt has been made to secure results further than those obtainable by simple agglutination tests and investigation of biochemical and morphological characters.

SOURCE OF SUPPLY.

The main source of strains for examination was the endemic area of Bengal. There had been established in Calcutta an Inquiry at the Campbell Hospital, in which special wards exist for the treatment of cholera, directed chiefly towards tests of the therapeutic value of bacteriophage, and in the course of the work all cases clinically resembling cholera which were admitted were submitted to routine bacteriological examination. From this source a large number of freshly isolated strains was available which included many inagglutinable strains. (The term 'inagglutinable' is applied in this communication to vibrios, which do not show the 'O' antigen of the typical *V. cholerae*.)

In the vicinity of Calcutta an area had also been selected for an Inquiry into the carrier problem and in this area with a population of about 10,000 routine systematic examination of healthy individuals was in progress. A large number of stools and water sources was examined and from these inagglutinable strains were also obtained. This area may be considered a typical endemic area.

Strains were also obtained from certain other areas.

Table XI shows that the chief sources other than Bengal are Mandapam, a quarantine station in the south of India through which emigrant labourers pass from Madras Presidency to Ceylon, and Rajputana, a dry hot area, where the incidence of cholera is in most years low, from which a series of water vibrios were collected by the authors. The latter sources consisted of jhils or lakes, rain-water ponds, wells in ordinary use, rivers, drains, taps, etc. The table shows sufficiently clearly the origin of the remainder of the strains.

In all a total of 558 vibrios has been dealt with. Of these 354 were isolated in the Bengal area and 204 from other sources. Two hundred and two of these were isolated from cases of clinical cholera, 182 from healthy persons and in occasional cases from those suffering from other diseases, and 172 from water sources. An approximately equal number of vibrios from the three main sources is therefore dealt with. Unfortunately the material available does not bear the same proportions in the endemic and non-endemic areas. A majority of case and carrier strains is examined from Bengal and a majority of water strains from other sources. The material isolated in Bengal in the epidemic year 1935-36 is recorded separately as there is available evidence of the epidemiological conditions prevailing during that year. The latter are considered in a separate paper.

In this investigation, although at least 1,000 different strains have been examined, only 558 are reported as, where biochemical and serological investigations have proved similarity between strains from the same source, whether case, carrier or water, duplicates have been excluded. With regard to case strains this means that the series of case vibrios from all sources, numbering 202, contains four examples in which two strains of different characters from one patient are included, one example in which three strains and one example in which four strains from one patient are included. The remainder represents one vibrio per patient. The position with regard to carrier strains is similar as there are very few strains of different types from the same individual. In the case of the water strains the position is different. Many strains from the same source are unclassified and therefore their serological character has not been worked out; amongst these it is probable that duplicates from the same source have been included. Amongst these, however, where serology and biochemical reactions have shown similarity in the one source, even though isolated at quite different periods, duplicates have been excluded.

SEROLOGICAL INVESTIGATION.

The strains submitted to serological investigation were those which did not agglutinate with a serum prepared against dried 'O' antigens of Inaba and Ogawa types issued from the Standards Laboratory, Oxford, for experimental trial. The further serological testing of these strains was carried out by means of 33 sera whose characters are given in Table I. These include:—

- (a) Type strain 'O' groups, Gardner and Venkatraman, II to VI—5 strains.
- (b) Selected non-agglutinating strains from cases of clinical cholera isolated in Calcutta—13 strains.
- (c) Selected case strains from other sources—8 strains.
- (d) Selected strains from Bengal carriers—4 strains.
- (e) Selected strains from Bengal water—2 strains.
- (f) Kohat water strain—1 strain.

In addition a serum was prepared against the rough variant of Inaba (Bruce White, 1935) and was used as a check on all vibrios. Any vibrios agglutinating with this serum have been excluded.

The technique employed is given in the Appendix.

Table II shows the results obtained. It will be seen that a number of definite serological groups has been obtained. The most striking fact, however, that emerges from a consideration of the tables is that, while 558 strains are listed, the largest serological group contains 40 vibrios only. There are two other groups of 40 and 30, two of 17, two of 16, one of 12, three of 11 and three of 10 and the remainder of the 31 groups are in single figures. In addition four of the sera used have agglutinated the homologous strains only. There is a certain amount of overlapping amongst the groups but each vibrio has been included in one group only. The table shows the strains Calcutta carrier 2898 and Calcutta case 324/7 in one group, as these were only different in having their own type specific antigen with a common group antigen. Shillong case strain 984 and Gardner's group IV are also included together as, although biochemically different, they are serologically very similar and agglutinate the same strains. In addition there was considerable relationship between Calcutta carrier 2943, Bengal water 1A, Calcutta case 1805/1, while Shillong case strain 3067 and Lahore case 10/5 have some points in common. Thus, although the grouping might be arranged to show one more large group serologically similar, the general finding is in any case the same that, in spite of the use of 33 sera, a maximum of four large groups has been obtained in 558 vibrios and the maximum percentage incidence of these groups is 7.2 per cent, while 247 vibrios remain unclassified.

The percentage incidence of the groups in the various sources is given in Table III. In view of the smallness of many of the groups the value of this table is diminished, but it shows that vibrios of even the smaller groups can be detected in more than one of the different sources, in spite of the very low percentage incidence that these groups may occupy on the whole. There is no group containing more than four vibrios that has not been found in at least two of the sources, while speaking generally those vibrios which are well represented in one source are also represented in at least one of the others. This is true not only for the vibrios as a whole, but also for the vibrios whose isolation dates and circumstances of isolation are known, as represented by the material collected in Bengal in the epidemic year 1935-36. The latter figures are also valuable in showing that there is no one particular period in which any one type is especially prevalent with the exception of 'Rangoon Rough'. In other words, no series of isolations of any size was due to any vibrio of one particular type.

Rangoon Rough represents 22.3 per cent of the inagglutinable vibrios isolated in the epidemic period, far the largest percentage incidence attained in the series. It has been isolated from cases in other areas than Bengal. It has been found on the same plate with the agglutinable vibrio on two authentic occasions. It has been isolated from at least three carriers and the classical Metchnikovi strain belongs to this serological type and is identical in its biochemical characters.

The percentage of vibrios classified is largest in 'cases' and less in 'carriers' and water and this applies also to the vibrios collected outside Bengal. Only five of the sera were prepared against carrier strains and of these two agglutinate only the homologous organisms: four of the sera were prepared against water strains. The remainder of the sera was against case strains. Two of the carrier sera agglutinate 17 and 11 vibrios respectively and that agglutinating 17 vibrios agglutinates two case strains only, while that agglutinating 11 does not agglutinate any case vibrio. One of the three water vibrio sera agglutinates no case vibrio

(group of 8) while the others agglutinate two and four out of a total of 12 and 11 respectively in the group. The proportion of case, carrier and water strains agglutinated by case vibrio sera is given in Table IIa. As the total number of case, carrier and water strains dealt with is approximately equal, it is clear from these facts that case sera have a closer relationship to case vibrios, while carrier and water sera have a lesser relationship to these vibrios.

From what has been said as to the method of selection of strains for inclusion in this report it will be clear that the percentage of strains classified is the minimum possible with the material received and the minimum claim is therefore put forward as to the classification obtainable. On the other hand, the classification is a purely serological one and some of the serological groups contain strains which give different biochemical reactions. The extent to which this has occurred can be gathered from Table IV, the findings of which will be commented on later. In addition, vibrios showing only 25 per cent of titre have also been included but only after a careful check of the findings. As the groups are not completely homogeneous it has not been thought worth while to separate these latter off, especially as their total number in the entire series is only 14 vibrios. It will be noted that from the 139 inagglutinable vibrio cases admitted to the Campbell Hospital in the epidemic year 1935-36, 112 vibrios have been investigated, representing 107 patients.

BIOCHEMICAL REACTIONS.

(a) *Fermentation tests.*—The fermentation reactions of all strains in this series were tested on mannose, saccharose and arabinose, the sugars which Heiberg (1935, 1936) has shown to be of special differential value in the examination of vibrios. In addition, glucose, lactose and mannite were also employed. The results of previous examinations of 494 vibrio strains from different sources, including both agglutinable and inagglutinable strains, had been reported (Taylor, Read and Pandit, 1936) and the finding of Heiberg that all agglutinable strains produced acid from mannose and saccharose but not from arabinose had been confirmed. In the examination of the present series of 558 strains which have also been submitted to serological examination, a classification of vibrios into the six fermentation types of Heiberg was carried out. One strain has been found of a seventh fermentation type. Taking the results with glucose and mannite, in addition to those with the three other sugars, it has been considered advisable to divide Heiberg type VI into two sub-types: sub-type 1, in which no sugar including glucose is fermented, and sub-type 2, in which glucose and mannite are fermented but not mannose, saccharose and arabinose. Strains belonging to these two sub-types, which differ in other biochemical reactions as well, would not have been differentiated if only the three essential sugars had been employed. Similarly within the types I and V, there were 11 and 3 strains respectively that did not ferment mannite; these have been separated and have hereafter been called aberrant types I and V. Nine out of the 11 aberrant type I strains and all the 3 strains of aberrant type V were Rajputana water strains. All the 10 strains of type VI, sub-type 1, were also water strains. Lactose was not fermented in 24 hours by any of the strains of the whole series except by two water strains. Glucose was fermented by all the strains except those of type VI, sub-type 1, and mannite was fermented by all except by type VI, sub-type 1, and the aberrant types I and V strains.

